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It is our great pleasure to present this Supplement Issue on “Macedonian Pharmaceutical Bulletin” to the scientific and professional community. This supplement includes the short communications accepted for the Seventh Congress of Pharmacy in North Macedonia with International participation 2020, which was postponed to 2021 due to the ongoing spread of Corona (SARS-CoV-2) virus over the world. Respecting the right of the authors to publish the results of their research, the Scientific Board decided to publish all papers that received a positive review, after obtaining the consent of the authors. The authors will also be invited to submit their papers in 2021, once the conditions for holding the Congress are provided.

The main theme of the Congress was “Modern trends in Pharmacy: opportunities and challenges” A broad spectrum of topics within the pharmaceutical sciences and practice carefully selected for this special occasion in order to build up a highly interesting and comprehensive program were covered. We received more than 120 short paper submissions from more than 15 countries. These numbers show that our Congress was aiming for the highest scientific standards, and that it can be considered a well-established venue for researchers in the broad fields of Pharmaceutical sciences and practice.

Sincere thanks to the hosts of the Seventh Congress of Pharmacy in North Macedonia with International participation, Macedonian Pharmaceutical Association and Faculty of Pharmacy, Ss Cyril and Methodius University in Skopje for their vision and commitments.

In the period the decision to postpone the Congress was made, organizational activities were at an advanced stage. We would like to thank the companies that showed interest in supporting our efforts during the organization. We acknowledge the sponsoring companies: the platinum sponsor AD ALKALOID, Skopje, the golden sponsors: PLIVA, EUROFARM, BOSNA LIJEK and KRKA, and the bronze sponsors: LEK, HEMOFARM and SEPTIMA.

We would also like to thank our members of the Scientific Committee for their volunteer time and dedication to the critical peer review process. We also wish to thank all the members of the Organizing Committee, whose work and commitment was invaluable.

On behalf of the Advisory and Scientific Committees, we would like to especially thank all internationally prominent researchers, whose work was supposed to be an essential part of the Congress. The interest in publishing their short communications in this issue of the Macedonian Pharmaceutical Bulletin is of a crucial importance for reinforcing the overall quality and standards of the bulletin. They give the state of the art of the recent advances in the field of pharmacy research.

The pharmaceutical sciences continue to grow as dynamic scientific interdisciplinary fields. We believe that published short communications will be an excellent source of scientific material in the fast evolving fields in Pharmaceutical sciences and practice.

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This issue of *Macedonian Pharmaceutical Bulletin* contains short papers accepted by the Scientific Committee for the presentation at the 7th Congress of Pharmacy in Macedonia with international participation 2020.

The authors are fully responsible for the contents of their short papers.

All reviewers that were involved in the short papers revision process are sincerely acknowledged.
The Medical Biochemistry and Laboratory Medicine are important diagnostic branches

Nada Majkić-Singh

Society of Medical Biochemists of Serbia, 11000 Belgrade, Serbia

Introduction

Medical biochemistry is the usual name for clinical biochemistry or clinical chemistry in Serbia, and medical biochemist is the official name for the clinical chemist (or clinical biochemist). This is the largest sub-discipline of the laboratory medicine in Serbia. It includes all aspects of clinical chemistry, and also laboratory hematology with coagulation, immunology, etc. Medical biochemistry laboratories in Serbia and medical biochemists as a profession are part of Health Care System and their activities are regulated through the Health Care Law and rules issued by the Chamber of Medical Biochemists of Serbia.

Since school-year 2006/2007 as a result of the Decision of the University Senate in Belgrade the Faculty of Pharmacy has been offering courses according to new curricula and syllabuses, entirely in the line with the Bologna Declaration, i.e. with the study programme of the EU member-states. The Bologna process represents a standardization of the European higher education area thus enabling compatibility and comparability of different study programme mobility of students and teaching staff as well as the possibility of degree recognition.

One of important attainments of the Bologna process is the European Credit Transfer System (ECTS). In order to meet the requirements of the National Accreditation Committee the adjusted study syllabuses have been adopted for the integrated graduate five year studies of Pharmacy and Pharmacy-Medical Biochemistry (Majkić-Singh, 2010).

Creative work and research in medical biochemistry requires broad formal training in basic natural sciences and medicine and extensive laboratory experience. During the studies Pharmacy-Medical Biochemistry, medical biochemist is qualifying for work in clinical-biochemical, toxicological and sanitary laboratory dealing with medical biochemical, toxicological and sanitary practice. In the course of education, a student is expected to acquire knowledge on: human organism, disease, role of biochemical laboratory in diagnostics and health care system. In the course of the study, a student is expected to acquire abilities and skills for: laboratory work, quality control that assures continuous process of checking and assessment measuring values to obtain reliable result and medicinally relevant information, handling with instruments and equipments, protection of laboratory staff and safe handling with chemicals and biological materials as well as pharmaceutical/medicinal waste (Majkić-Singh, 2011; Study Programme).

EFLM syllabus for postgraduate education and training for Specialist in Laboratory Medicine

Although laboratory medicine practice varies across the European Union’s (EU) member states, the extent of overlap in scope is such that a common syllabus describing the education and training associated with high-
quality, specialist practice can be identified. In turn, such a syllabus can help define the common set of skills, knowledge and competence in a Common Training Framework (CTF) for non-medical Specialists in Laboratory Medicine under EU Directive 2013/55/ EU (The recognition of Professional Qualifications). In meeting the requirements of the directive’s CTF patient safety is particularly enhanced when specialists seek to capitalize on opportunities for free professional migration across EU borders. In updating the fourth syllabus, the fifth expands on individual discipline requirements, new analytical techniques and use of statistics. An outline structure for a training programme is proposed together with expected responsibilities of trainees and trainers; reference is provided to a trainee’s log book. In updating the syllabus, it continues to support national programs and the aims of EU Directive 2013/55/EU in providing safeguards to professional mobility across European borders at a time when the demand for highly qualified professionals is increasing in the face of a disparity in their distribution across Europe. In support of achieving a CTF, the syllabus represents EFLM’s position statement for the education and training that underpins the framework (Jassam et al., 2018).

A syllabus is a plan showing the subjects and/or books to be studied in a particular course, especially a course that leads to an examination. When there is substantial similarity between syllabi, it opens the opportunity to harmonise common principles of education and training. The transposition of European Union (EU) Directive 2013/55/EC (The Recognition of Professional Qualifications) into member states’ national laws in January 2016 reflected that although there is demand for highly qualified individuals across the Union, there is also disparity in their distribution. In creating a mechanism for mutual recognition of professional qualifications, the directive supports individuals seeking unhindered free professional movement across EU borders, helps catalyze a more equitable distribution of human resource and services across the Union, can obviate the need for member states to impose “compensation measures” (e.g. retraining, new qualifications, aptitude tests and adaptation periods) on each other’s professionals, which may needlessly delay and deter migration.

Throughout training, the objective is to develop the knowledge, skills, competence, attitudes and behaviors consistent with specialist level clinical, scientific and professional practice. Clinically, the specialist assesses the appropriate clinical investigations for his/her local population; evaluates how those investigations relate to diagnosis, management and prognosis; and provides the expertise to ensure appropriate application. Scientifically, he/she is able to assess the scope of service need, plan and implement its delivery and ensure a safe and effective working environment. Professionally, the specialist is able to take personal responsibility for his/her actions, working autonomously to take the initiative in complex and unpredictable situations. Additionally, specialists assess, plan, conduct, report, diffuse and adopt their research, development and innovation output. Their clinical leadership training contributes to the evolution of health and healthcare services (Jassam et al., 2018).

References


Study Programme: Master of Pharmacy-Medical Biochemist, University of Belgrade, Faculty of Pharmacy, Belgrade. Available at: www.pharmacy.bg.ac.rs.
Therapeutic drug monitoring of valproic acid through plasma concentration

Verica Jakjimoska\textsuperscript{1,*}, Biljana Gjorgjeska\textsuperscript{2}

\textsuperscript{1} General City Hospital “8th September”, Central Biochemical Laboratory, Pariska bb, 1000 Skopje, N. Macedonia
\textsuperscript{2} Faculty of Medical Science, University “Goce Delcev” Stip, Krste Misirkov 10A, 2000 Stip, N. Macedonia

Introduction

Therapeutic drug monitoring is the measurement of specific drug and/or their breakdown products (metabolites) at timed intervals to maintain a relatively constant concentration of the medication in the blood. Some of the monitored drugs tend to have a narrow “therapeutic index”, which is a ratio between the toxic and therapeutic (effective) dose of medication.

Burtn et al. (2006) have shown that valproic acid is an 8-carbon 2-chain fatty acid that is metabolized by the liver and processed at a variable rate based on the patient’s liver function and age, in addition to patient’s other routine medications with which valproic acid may interact. At therapeutic concentrations, valproic acid mediates prolonged recovery of voltage-activated Na\textsuperscript{+} channels, thereby inhibiting repetitive firing induced by depolarization of cortical and spinal cord neurons.

Unborn babies exposed to valproate are at very high risk of neurodevelopment disability and other birth defects and the need for effective contraception planning must also be emphasized, along with the requirement for specialist oversight to safely change their medication if planning a pregnancy according to Marshall and Bangert (2008).

The determination of an antiepileptic drug concentration is recommended as a baseline measurement after starting drug therapy, according to NICE Clinical Guideline 137 (2020), after a change in the drug dose regimen, after addition of a second drug that may interact with the antiepileptic drug, and after a change in the patient’s liver, cardiac, or gastrointestinal function.According to NICE Clinical Guideline 38 (2020), measurement of an antiepileptic drug concentration is usually performed after 4 to 5 elimination half-lives of drug administration, that is, once a steady state concentration has been achieved.

Materials and methods

This is a retrospective study on 40 patients over the period from January the 9\textsuperscript{th} to March the 11\textsuperscript{th} 2020. All serum samples were selected from specimens submitted to the laboratory for routine antiepileptic drug testing for valproic acid. The analyses were performed using an automated analyzer Immulite 2000, competitive immunoassay methods for the quantitative measurement of valproic acid. The Immulite 2000 is an automated random access analyzer that uses chemiluminescence technology.

The therapeutic range for valproic acid is 50-100 mcg/mL, sub-normal level is < 50mcg/mL, and the toxic level is > 100 mcg/mL.
**Result and discussion**

In this study were analyzed 40 patients coming from the department of neurology and psychiatry, patients with epilepsy or with bipolar mood disorders and other neuralgia. Males represented 37.5% of the total number of patients, and females 62.5%, which is 25% more than males, showing that females are more vulnerable. Standard deviation for patient’s age within this population with 95% confidence interval was 45.9±5.4 years. Female only, has shown lower age standard deviation with 95% confidence interval of 32.5±2.5 years. Approximately 42.5% of the population were with normal serum level of valproic acid (50-100 mcg/mL), and 57.5% were with sub-normal serum level of valproic acid (< 50mcg/mL), and none with toxic level of valproic acid (> 100mcg/mL) in serum samples. Within 95% confidence interval, serum levels were measured to be 46.4±7.5 (±16%) mcg/mL. Mean of inter-individual variability in valproic acid serum concentrations were 38.9% CV for dose of 400mg/day, 45.8% CV for dose of 600 mg/day, and 14.25% CV for dose of 800 mg/day.

As demonstrated (Warner et al., 1998), many of the drugs that require therapeutic monitoring are taken for a lifetime. They must be maintained at steady concentrations year after year while the person ages and goes through life events that may alter that individual's therapeutic level, including pregnancies, temporary illnesses, infections, emotional and physical stresses, accidents, and surgeries.

Tests that measure drug concentration in serum are used to monitor blood levels of drugs that have a narrow range in which the drug is effective, but not toxic. It was shown that no toxicity was found and therapeutic response was satisfying within 42.5% of the patients. There is significant number of patients (57.5%) with sub-normal serum level of valproic acid, partially because this low level has satisfactory therapeutic response, but there is still chance for non-compliance. In addition, Shaikh (2018) demonstrated that some drugs require monitoring because the dosage of drug given does not correlate well with the concentration of drug that may reach the blood. Sometimes, the way that a drug is absorbed and metabolized can vary from person to person, or the physical or health status of a person can affect the drug level in the blood. Through years of testing, the optimum therapeutic ranges for drugs have been determined. In these ranges, most people will be effectively treated without excessive side effects or symptoms of toxicity.

**Conclusion**

Patients treated with valproic acid are most likely woman at the age of 30-35 years, still in reproductive period and have childbearing potential. This fact requires protection for girls and young women, and using valproic acid only when other medications have not been tolerated or have been found to be ineffective. It is vital where valproate is prescribed to girls and women of childbearing potential that they are made aware of the risks of taking the medication in pregnancy. Sub-normal level of valproic acid in serum is partially because this low level has satisfactory therapeutic response but there is still chance for non-compliance.

**References**


In vitro antimicrobial properties of basil and thyme essential oils against Salmonella Spp.

Metodija Trajchev¹*, Jasmina Stojiljkovic², Dimitar Nakov¹, Marija Glavash Dodov³, Milena Petrovska⁴

¹Faculty of Agricultural Sciences and Food, Ss. Cyril and Methodius University, Blvd. Aleksandar Makedonski bb, 1000 Skopje, North Macedonia
²College of Applied Studies, Filip Filipovic 20, 17500 Vranje, Republic of Serbia
³Faculty of pharmacy, Ss. Cyril and Methodius University, Majka Tereza 47, 1000 Skopje, North Macedonia
⁴Faculty of medicine, Ss. Cyril and Methodius University, 50 Divizija 6, 1000 Skopje, North Macedonia

Introduction

Food diseases are caused by consuming foods that have been contaminated by an infectious agent or a toxin produced by it. According to the WHO, 30% of people in industrialized countries suffer from foodborne diseases. Salmonella enteritidis even at 95.9% was the main etiological factor for salmonellosis in humans (De Knecht et al., 2015) and have established a trend of increasing the level of contamination of food products with Salmonella spp. in the Republic of North Macedonia. In order to protect food from contamination with pathogens and other harmful microorganisms, many scientists have examined the antifungal, antibacterial and antioxidative properties of essential oils (EOs) and their application in food technology. They are considered a safe and environmentally friendly alternative to the control of bacteria present in the food and food industry, but also to the control of other pathogenic microorganisms, especially those that are drug resistant (Yap et al., 2014).

The aim of the study was to determine the antimicrobial effect of different concentrations of basil and thyme EOs on the growth of Salmonella enteritidis in laboratory conditions, inoculated in dough for making pasta with eggs and inoculums with flour and chicken egg.

Materials and Methods

All samples were prepared in duplicate. The test samples were prepared as micellar solution from basil and thyme EOs in physiological solution (PhS) up to final concentration of 1, 2.5 and 5%, as well as PhS without EOs as a control. The first group of test samples were prepared from 25 g of the dough used for preparation of the egg-based pasta, inoculated with 0.1 mL of the suspension of Salmonella enteritidis with the initial number of bacteria from $10^9$ CFU/g. After inoculation, the micellar solutions of basil and thyme EOs were added to the prepared dough. A quantity of 25 g of dough was mixed with 225 mL of selenite F broth and incubated for 24 h at 37 °C.

The second group of test samples was prepared as a mixture of flour and a mixture of egg with the micellar solutions of basil and thyme EOs. Afterwards, the samples were inoculated with Salmonella enteritidis. The ready suspension from Salmonella enteritidis have been inoculated in 5 mL of the micellar solutions of basil and thyme EOs as well as 5 mL of PhS (control), in initial

* metot@fznh.ukim.edu.mk
concentration of bacteria from $10^9$ CFU/mL. In the samples, 90 ml of Salenit F broth were added and duplicate samples were incubated at two regimes: one sample for 9 h at 46 °C and the other one for 18 h at 37 °C. Dilutions 1:20 and 1: 200 were prepared from all samples and from them 0.1 ml was inoculated on Müller-Hinton agar, for enumeration of bacterial cell count (CFU). Petri plates were incubated at 37 °C for 18 hours (ISO 6579-1, 2017). Data analysis was carried out with GLM-General Linear Model.

**Results and discussion**

Foods of animal origin, especially poultry and poultry products, including eggs, have been consistently implicated in sporadic cases and outbreaks of human salmonellosis (FAO/WHO, 2002). Hence, the use of plant EOs as a substitute for synthetic antimicrobials is of great importance as most of them have proven efficacy in the control of bacteria present in the food and food industry.

Related to the results from the research, the Log$_{10}$ number of bacterial cells of *Salmonella enteritidis* was gradually decreased in each successive concentration of basil and thyme EOs. In the samples of pasta dough with micellar solution of basil EOs, the CFU of *S. enteritidis* showed a trend of decreasing from the samples without added EOs to the samples with added 5% of basil EOs, ranged from 8.46±0.071 to 8.04±0.113 Log$_{10}$ CFU/mL. In the samples of dough with micellar solution of thyme EOs, the decreasing ranged from 8.24±0.075 to 7.29±0.142 Log$_{10}$ CFU/mL. The same trend was established also in the samples with a mixture of flour and homogenized egg. The decreasing in the Log$_{10}$ CFU/mL of *Salmonella enteritidis* in the samples with added basil EOs was ranged from 5.69±0.700 to 3.08±0.805; while in the samples with thyme EOs from 5.72±0.064 to 1.84±0.784 Log$_{10}$ CFU/mL *Salmonella enteritidis*, respectively. Overall, in all samples the trend of decreasing in the Log$_{10}$ number of *Salmonella enteritidis* from control samples to the samples with added 5% EOs ranged from 7.77±0.184 to 5.93±0.407 Log$_{10}$ CFU/mL. The GLM statistical model showed that the Log$_{10}$ number of bacterial cells of *Salmonella enteritidis* was statistically significant influenced by the added EOs (F= 4.171; df=1; p<0.05) and their concentration (F= 4.879; df=3; p<0.01), while their interaction didn’t show statistically significant influence (F= 0.350; df=3) on *Salmonella enteritidis* Log$_{10}$ CFU/mL.

Herewith, the findings of some authors suggest that the reason for the weaker antimicrobial effect of plant EOs, is the interaction that have essential oils and bacteria with the food ingredients (Hayouni et al., 2008). It has been found that in order to obtain the optimal antibacterial effect in the food, it is necessary to use several times higher concentration of EOs of plants than the laboratory determined concentration (de Oliveira et al., 2013).

**Conclusion**

The basil and thyme EOs as natural preservative might be useful in the control of *Salmonella spp.* in food like pasta, prepared from white flour and eggs, as an alternative of the chemical preservatives.

**References**


Protective role of sulforaphane against phthalate and bisphenol A mixture linked hepatocellular carcinoma: in silico toxicogenomic datamining

Katarina Baralić*, Katarina Živančević, Dragica Jorgovanović, Dragana Javorac, Evica Antonijević, Aleksandra Buha Djordjevic, Marijana Ćurčić, Zorica Bulat, Biljana Antonijević, Danijela Đukić-Ćosić

Department of Toxicology „Akademik Danilo Soldatović“, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11000 Belgrade, Serbia

Introduction

Phthalates and bisphenol A have been widely used as plasticizers in many consumer products. In addition to acting as endocrine disruptors, recent studies indicate that these substances promote the progression of several types of cancers, including hepatocellular carcinoma (HCC) (Tsai et al., 2015; Weinhouse et al., 2014). The protective effect of many substances on hepatocellular carcinoma has been investigated in recent years. Sulforaphane (SFN), a very potent isothiocyanate isolated from broccoli, was found to be the most potent naturally occurring inducer of phase 2 enzymes, known to lower susceptibility to chemical carcinogenesis (Priya et al., 2011).

By integrating measuring families of cellular molecules with bioinformatics and conventional toxicology, toxicogenomics can be useful for exploring interactions between genes and chemicals in disease causation, as well as for mixture toxicity assessment and generating hypothesis on chemical therapeutic and protective actions (Boverhof and Zacharewski, 2006).

Thus, the aim of this study was to explore the link between the mixture of two phthalates (bis (2 – ethylhexyl) phthalate (DEHP) + dibutyl phthalate (DBP)) and bisphenol A (BPA) and HCC development by using toxicogenomic data mining in silico analysis. Furthermore, this study aims to investigate the ability of sulforaphane to elucidate toxic effects of the investigated mixture on the gene level.

Materials and methods

The Comparative Toxicogenomic Database (CTD; http://ctd.mdibl.org) was used as the main data mining tool in this research. Batch Query and Set Analyser CTD tools were used to retrieve all curated HCC – gene interactions for the investigated substances, as well as the molecular pathways these genes were involved in. MyVenn CTD tool was used to find the genes common to all the investigated substances. GeneMania prediction server (https://genemania.org) was used for constructing tight network of genes related to the HCC development. GeneCards: the Human Gene Database (https://www.genecards.org) was used for browsing the genomic, transcriptomic, proteomic, genetic, clinical and functional information about the selected genes.

* katherinekatabarka@gmail.com
Results and discussion

SetAnalyser CTD tool revealed that DEHP, DBP and BPA interact with 183, 222 and 480 HCC related-genes, respectively, all of them listed as HCC marker/mechanism genes. Likewise, SFN interacted with 70 HCC-related genes. However, unlike DEHP, DBP and BPA, it was marked as possible therapeutic agent for HCC.

MyVenn CTD tool revealed the common genes for the investigated substances. DEHP, DBP and BPA interacted with 111 common HCC genes, while 40 of them matched with SFN associated genes. Interactions of the three investigated toxic substances with 14 out of the 40 genes matched (ACTB, CASP8, CCNB1, CYP1A2, CYP2E1, DCN, DPYD, EPHX1, IL6, IRS2, MYC, NFE2L2, PGD, SOD2), while SFN interacted with all of these genes in an opposite manner. GeneMania prediction server was used to construct tight network of the 14 highlighted genes, together with 20 related genes. There was a total of 136 links between these genes. More than half of them (59.51%) were in co-expression, while 21.45% were genetic interactions.

Set Analyzer CTD tool (p-value - 0.01) listed 6 enriched pathways these 14 genes were involved in: FoxO signaling pathway, non-alcoholic fatty liver disease, metabolism of xenobiotics by cytochrome P450, chemical carcinogenesis, phase 1 - functionalization of compounds, hepatitis B.

GeneCards database was used to further explore the functions of 14 selected genes (Stelzer et al. 2016). DEHP, DBP and BPA decreased the expression of CASP8 mRNA (gene involved in apoptosis and control of the cell growth) and CCNB1 (gene responsible for control of the cell cycle), while SFN acted oppositely. Furthermore, while DEHP, DBP and BPA decreased the expression of genes involved in the metabolism of xenobiotics and chemical carcinogenesis (CYP1A2, CYP2E1, EPHX1), SFN increased their expression. As for the tumor suppressor genes, DEHP, DBP and BPA decreased the expression of DCN mRNA, while SFN increased its expression. Furthermore, DEHP, DBP and BPA activated proto-oncogene MYC, while SFN inhibited its action.

Conclusion

Our toxicogenomic in silico data mining identified 14 common HCC-related genes for DEHP, DBP and BPA. Sulforaphane interacted with all of these genes in an opposite manner, indicating a possible protective effect that should be confirmed by further in vitro and in vivo studies.

References


The ameliorative effect of bioactive phytochemicals (resveratrol, curcumin and sulforaphane) on environmental chemicals evoked inflammation: toxicogenomic data mining approach

Katarina Živančević*, Katarina Baralić, Dragica Jorgovanović, Danijela Đukić-Ćosić

Department of Toxicology „Akademik Danilo Soldatović“, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11000 Belgrade, Serbia

Introduction

Inflammation is a well-described adaptive response triggered by endogenous and exogenous antigens and different conditions, such as infection and tissue injury. However, wide varieties of physiological and pathological processes are connected with inflammation. We distinguish acute inflammation, which is manifested by cardinal signs of inflammation, and chronic inflammation that can occur in the absence of some of them (Medzhitov, 2008; Khansari et al., 2009).

The low-grade systemic chronic inflammation can contribute to emergence of serious pathological conditions such as autoimmunity, neurodegeneration, diabetes, cancer and accelerated aging. Although usually inflammation is not a primary cause, it plays an important role in development of these diseases, and treatment focused on suppression of inflammatory reactions and its consequences in many cases can ameliorate these conditions (Kuprash and Nedospasov, 2016).

There is a growing body of evidence demonstrating that environmental chemicals promote different diseases by inducing inflammation (Ghezzi et al., 2018).

Conversely to inflammatory-promotive mode of action, resveratrol, curcumin and sulforaphane are powerful phytochemicals known for their antioxidant and anti-inflammatory properties, thus they can be examined as a potential protective combination in the treatment of inflammation (Chen et al., 2018).

The aim of this in silico study was to analyze the individual and combined therapeutic effects of resveratrol, curcumin and sulforaphane on the regulation of genes associated with the development of inflammation evoked by environmental chemicals using the toxicogenomic data mining approach.

Materials and methods

The Comparative Toxicogenomic Database (CTD; http://ctd.mdibl.org) and its tools (Batch Query, MyVenn and Set Analyzer) were used to obtain the information about the interactions of investigated phytochemicals with genes/proteins associated with environmental chemical-linked inflammation. Functions of genes were obtained from the GeneCards: the Human Gene Database (https://www.genecards.org), while GeneMania prediction server (https://genemania.org) revealed detailed gene interactions.

* kajaziv93@gmail.com
Results and discussion

Inference Score, representing a measure of degree of support for a given association between a disease and a chemical, showed that bisphenol A, cyclophosphamide, trinitrobenzene sulfonic acid, ferrous sulfate, ozone, acrolein, toluene 2,4-diisocyanate, asbestos, hydrochloric acid, and pyruvaldehyde have the strongest connectivity with inflammatory processes development among all environmental chemicals listed in the CTD. Additionally, CTD contains list of chemicals marked as therapeutic agents for inflammation. In our study we have focused on protective properties of bioactive phytochemicals (resveratrol, curcumin and sulforaphan). Resveratrol curcumin and sulforaphan interacted with 87, 60 and 31 genes that showed suppressive effect on inflammation, respectively (Batch Query). MyVenn CTD tool revealed that resveratrol, curcumin and sulforaphan interacted with 26 common genes which show inflammatory suppression (ADIPOQ, AGT, AHR, AKT1, BDNF, CCL2, CXCL8, HMOX1, ICAM1, IFNG, IL1B, IL6, JAK2, KNYU, MMP2, NFG, NOS2, PARP1, PPARG, PTGS2, SOD1, TFRC, TGFβ1, TIMP1, TLR4, TNF). They participate in 60 different metabolic pathways associated with the modulation of inflammatory response (Set Analyzer), including interleukin signaling pathway (IL-4, IL-6, IL-10, IL-13, IL-17), HIF-1 signaling pathway, NF-kappa B signaling pathway, TNF signaling pathway, MAPK signaling pathway, Jak-STAT signaling pathway, PI3K-Akt signaling pathway, signal transduction, adipocytokine signaling pathway that affect a wide range of biological processes, such as cell growth, cell cycle, transcription, cytoskeletal redistribution, cell proliferation, differentiation and apoptosis.

Ghezzi et al. (2018) have shown that the immune system plays an important role in inflammatory response and cancer pathways through toll-like receptor signaling pathway (a particularly important is TLR4). The investigated dietary phytochemicals act anti-inflammatory by modulating immune system through toll-like receptor signaling pathway (AKT1, CXCL8, IL1B, IL6, TLR4, TNF) and cytokine signaling in immune system (AKT1, CCL2, CXCL8, HMOX1, ICAM1, IFNG, IL1B, IL6, JAK2, MMP2, NOS2, PTGS2, TGFβ1, TIMP1, TNF). GeneMania server revealed that most of common genes were in co-expression - simultaneous expression of genes (68.38%), co-localisation - genes found in the same location (17.22%) and physical interaction - protein-protein interactions (8.88%).

Conclusion

These results confirm both individual and combined anti-inflammatory effect of investigated phytochemicals that could be considered for further in vitro and in vivo investigation in order to clarify the mechanisms of their beneficial effects, especially the different acting manner of their common 26 genes.

References


Enteral nutrition in Macedonian hospitals

Elena Karabeleski\(^1\)*, Lidija Petrushevska-Tozi\(^2\), Emilija Kostoska\(^3\), Irena Radivojsha\(^4\), Kristina Mladenovska\(^2\)

\(^1\)Evropa Lek Pharma DOOEL, St Jadranska Magistrala No. 31, 1000 Skopje, N. Macedonia
\(^2\)Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa No. 47, 1000 Skopje, N. Macedonia
\(^3\)Zan Mitrev Clinic, St. Bled Agreement No. 8, 1000 Skopje, N. Macedonia
\(^4\)PHO City General Hospital 8th September, St. Bled Agreement NN, 1000 Skopje, N. Macedonia

Introduction

Nutrition products that are delivered via the gastrointestinal (GI) tract as tube feeding are a valuable option for improving organ function and immune-competence, aiding in recovery process and reducing the convalescence period. Enteral nutrition (EN) is indicated in unconscious patients, disease-related malnutrition, anorexia, upper GI obstruction or dysfunction, malabsorption, stroke, etc. EN is recommended even in patients without obvious undernutrition, if the patient will be unable to eat for more than 7 days preoperatively. It is also recommended for patients who are not able to maintain oral intake above 60% of recommended intake for more than 10 days. In addition, delay of surgery for preoperative EN is recommended for patients at severe nutritional risk, with at least one of the following: weight loss > 10–15% within 6 months, BMI < 18.5 kg/m\(^2\), serum albumin < 30 g/L (with no evidence of hepatic or renal dysfunction).

The benefits from EN include: better clinical outcomes; reduction of hospitalizations, complications and deaths; improved quality of life and healthcare savings (Weimanna et al., 2006).

Limited hospital budgets, shortage of trained medical workers and lack of clinical nutrition protocols limit the use of EN in hospitals. The aim of this study was to demonstrate the availability and use of EN in the Macedonian hospitals. In addition, nurses’ perceived barriers for utilizing EN were explored.

Materials and methods

Commercially available EN formulas registered on the Macedonian market were identified by inspection in the Register of Macedonian Food and Veterinary Agency (www.fva.gov.mk/mk/registriran-nezivotinsko-poteklo). Similarly, the data from the Public Procurement Bureau (www.e-nabavki.gov.mk/PublicAccess/home.aspx#/notices) were used to identify the number of enteral food units that were procured by the public hospitals. The data for the average annual number of patients in public hospitals, with average days of hospital stays were extracted from the Macedonian Health Insurance Fund (www.fzo.org.mk/WBStorage/Files/Godisen%20Izvestaj%202017.pdf). Also, a validated questionnaire was used to identify the barriers that hinder nurses from utilizing effective practices of EN. The questionnaire was divided into 6 subscales, including the guideline recommendations and implementation strategies (I), delivery resources (II), dietitian support (III), delivery of EN to the patient (IV), care provider
attitudes and behaviors (V) and economic impact of enteral nutrition for hospitals and patients (VI).

Descriptive statistics was used to answer research questions. The respondents (nurses) from public and private hospitals were asked to rate the barrier using five-level Likert scale, from 1 (strongly disagree) to 5 (strongly agree). Data were presented as frequencies and percentages for categorical variables while means and standard deviations were used for continuous variables. A series of independent sample t-tests and analysis of variance (ANOVA) were used to describe differences in the perceived barriers of EN. A total of 50 nurses from four public (74%) and one private hospital (26%) were recruited, 32% with a bachelor degree and between 1 and 36 years’ experience in nursing.

Results and discussion

EN formulas are registered on the Macedonian market, including polymeric formulas, which contain whole proteins and are intended for patients with normal digestion and absorption (300–500 mOsm/kg, 1–1.2 kcal/mL, 30–40 g protein/L); semi-elemental peptide feeds, indicated for patients with disputed GI function, who need partially hydrolyzed nutrients for better digestion and absorption (osmolarity depends on the level of hydrolysis, 1–1.2 kcal/mL, 30–45 g protein/L); and disease specific enteral formulas, designed for specific clinical conditions. In addition, EN formulas from 0-1 year, 1-8 years, < 1 year and adult formulas are also available.

From 55 public hospitals in total (27 university clinics, 3 public clinical, 9 special, 13 public general and 3 psychiatric hospitals), in only 20% of them EN was administered to the patients, in limited quantities. The annual number of patients in public health institutions was 208,997 in 2017, with average hospital stay of 5.5 days, while the number of EN bottles planned for purchasing 7,680 bottles of 500 mL/500 kcal. Having these in regard as well as the number of patients admitted to surgical units in 2017 (72,026 patients), their average hospital stay (4.45 days) and daily intake of EN 1000 kcal, to 1% of all hospitalized patients, EN was administered.

Percent of answers ranged between 98-100%. Majority of respondents in the public hospitals were undecided/neutral for most of their responses (mean±SD I: 3.40±1.12, II: 3.58±0.95, IV: 3.48±0.84, V: 3.96±0.61), disagreeing that the EN is significant financial burden for the hospital and patients (mean±SD VI: 2.54±0.74) and that the dietitian support is sufficient for providing EN and monitoring and evaluation of EN outcomes (mean±SD III: 2.39±0.84). No significant difference in majority of responses was observed between the nurses employed in public and private hospitals. However, statistically significant difference was obtained when the support of dietitian was compared (mean±SD III: 2.39±0.84 vs. 3.26±1.23, accordingly, p=0.0067). As most important barriers for public hospitals, lack of dietitian support was emphasized i.e. need of one dietitian at least, with full working hours (incl. night shifts, weekends and holidays), educated to provide nutrition screening for risk of malnutrition for each hospitalized patient, to monitor and evaluate outcome of EN and provide training for patients how to administer EN after discharge.

Conclusion

The needs of screening for nutritional risk in hospitals and early nutritional protocols have been already recognized, having in regard that one in four patients admitted to hospital have disease-related malnutrition and about 30% of all hospitalized patients are undernourished or their undernutrition develops further when admitted to hospital (Kondrup et al., 2003). Participants in this study moderately perceived barriers for EN in Macedonian hospitals, with more focus on dietitian support. This barrier is modifiable and can be managed by setting a multi-disciplinary team in the hospitals, with a dietitian specialized for EN, and by education for clinical nutrition as a part of multidisciplinary approach in the treatment of hospitalized patients.

References


Regulatory status of nutraceuticals – is it rationale to rethink?

Tanja Petreska Ivanovska*, Zoran Zhivikj, Lidija Petrushevska-Tozi

Institute of Applied Biochemistry, Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia

Introduction

The spread of knowledge related to the impact of diet and specific food components have allowed designing healthy food contributing to improved health and overall well-being as well as reduction of chronic diseases. Many studies are focused to an understanding of the potential mechanisms of action of pharmaceutically active substances contained in food. This category named “functional food” or “nutraceuticals” represents a modification of traditional food and it has been confirmed to have a beneficial effect on health beyond nutrition (Corbo et al., 2014). A growing demand for these products which may help to prevent the onset of pathological conditions require proper assessment of their safety, mechanism of action and efficacy with pre-clinical and clinical studies. However, there are specific legislation of nutraceuticals in different countries experiencing challenges with safety and health claim substantiation. Bridging the regulatory gaps by creating an equivocal international regulation is of utmost importance and can help the grey area between pharmaceuticals and food to be overcome.

Current regulations

The concept of functional foods was first developed in Japan in the 1980s. This concept defined the functional food as the food eligible to reduce the risk of specified health concerns. In 1991, the Japanese Ministry of Health and Welfare launched the first legalization of functional foods under the term “Foods for Specified Health Uses” (FOSHU). In 2001, the Japanese government adopted new regulation system “Foods with Special Dietary Declarations” divided in two categories: Food with nutrient function claims which may contain vitamins and/or minerals and can be distributed without special registration or notification of relevant authorities and FOSHU or food that contains ingredients with health-promoting properties officially recognized to have a positive physiological effect on the human body (Shimizu, 2003). In the EU, the concept of functional foods was firstly introduced within the framework of the research project “Functional Food Science in Europe” (FUFOSE). According to this, food may be considered functional if beneficial effect on one or more functions of the organism beyond its nutritional effect was proven resulting in improve health and well-being and/or reduce the risk of diseases. Functional foods must resemble to conventional foods and be a part of a normal diet showing their beneficial effects when consumed in normal amounts with the diet. In the EU functional food can be classified according to the Regulation (EC) 258/97 of the European Parliament and of the Council on novel foods and novel food ingredients as well by using a Regulation (EC) 1924/2006 on

* tpetreska@ff.ukim.edu.mk
nutrition and health claims made on foods. The last one was issued to harmonize different legislations among Member States to guaranty safety and efficacy and to protect the internal market by providing proper information to consumers. Herein, any nutrition or health claim made on food must be scientifically evaluated and approved by the European Food Safety Authority (EFSA) (Bagchi, 2014). These general principles which are also acquired in the legislation of North Macedonia, apply to all foods (functional foods, food supplements, herbal products, probiotics and prebiotics and dietetic foods) for which a statement that the foodstuff contains a beneficial nutritional or physiological substance. While EFSA must authorize in detail any health claim before it is considered at a national or European level prior the new product reaches the market, the FDA in USA is authorized to act against any unsafe product launched on the market (Santini et al., 2018).

In accordance to US regulation, only nutrition and health claims approved by the FDA can be used. The approval by FDA is given in a case the statement complies with safety aspects of a set of regulations different from those of conventional foods and drugs. According to Dietary Supplement Health and Education Act (1994), manufacturer is responsible regarding the safety of the nutraceutical before it is marketed. Apart the food manufacturers, nutrition and health statements may be requested by organizations that promote healthy diet, protect the right of consumers or either consumers themselves. Food and Drug Administration Modernization Act (1997) enabled health and nutrition claims to be authorized based on an authority’s statement from the Academy of Sciences or other federal authorities after notifying the FDA at least 4 months before introducing the product on the market. Another legal statement is structure or functional claims which do not refer to diseases, but describe the effect of a component on the structure and function of the body or general well-being. The requirements for these statements are only to be true and do not be misleading or confusing without necessity to be authorized by FDA (Bagchi, 2014).

Future considerations

The lack of a shared legislation is a big challenge for nutraceuticals globalization because the existence of different legislations can generate confusion resulting in dissimilarity in definition of the same type of product in different countries, leading to confusion among consumers, and eventually to possible misuse. Comprising that no completely effective regulatory system exists in Europe or in the USA, it would be helpful to define nutraceuticals in a new category that differentiates them from food supplements and pharmaceuticals (Santini et al., 2018). In Japan, functional foods are defined according to their use of natural ingredients, whereas in the US, they can also contain ingredients produced with biotechnology, thus confusing information may be the administration of the pharmaceutical form which can be the same for food supplements and nutraceuticals (Santini et al., 2018). The updated European Regulation 2015/2283 define food categories including a definition of food supplements, but EFSA still does not make any distinction between food supplements and nutraceuticals for health claim application on new products. Consumer’s needs to create a clear perception also enforced more consistency regarding the regulatory system of nutraceuticals. A uniformly developed regulation system for identification and classification of nutraceuticals at an international level including reliable assessment of quality, efficacy, mechanism of action and safety could potentially benefit both the consumers and the industry.

References

Effects of L-2-Oxothiazolidine-4-carboxylate on isoproterenol-induced acute myocardial infarction in rats

Marija Angelovski*, Dino Atanasov, Mitko Mladenov, Nikola Hadzi-Petrushev

Faculty of Natural Sciences and Mathematics, Arhimedova 3, 1000 Skopje, Republic of North Macedonia

Introduction

Myocardial ischemia occurs when myocardial oxygen demand exceeds oxygen supply leading to myocardial infarction (MI), which is one of the most lethal manifestations of cardiovascular diseases. Reactive oxygen species (ROS) and the associated oxidative stress are contributors to the pathological changes following MI (Lefer and Grabger, 2000). Isoproterenol (ISO), a synthetic catecholamine and beta-adrenergic agonist, induces oxidative stress in the myocardium, which may be a causative factor for irreversible damage of the myocardial membrane (Suchalatha and Shyamala, 2004). To prevent the damage by ROS, various antioxidant defense systems are present in the myocardial tissue and the glutathione/glutathione peroxidase system appears to be the main and the most active mechanism (Singh et al., 1989). The depletion of the antioxidant mechanisms renders cells particularly vulnerable to oxidative stress. Hence, the enhancement of the myocardial glutathione system might have protective effects.

Considering the limited effectiveness of the direct administration of glutathione, and the advantages of L-2-Oxothiazolidine-4-carboxylate (OTC) in enhancing the glutathione levels compared to other prodrugs (Cacciatore et al., 2010), this study examined the beneficial effects of OTC supplementation during the development of MI, by evaluating the cardiac glutathione content, the markers for cardiac lipid and protein oxidation, and the histopathology of the myocardium.

Materials and methods

Animals and experimental design

Male Wistar rats (200-250 g BW, n=32) were divided into four equal groups. The rats in the OTC and in the OTC+ISO groups were treated with OTC (6.5 mmol/kg BW, i.p.) twice a day for two consecutive days. Rats from the groups ISO and OTC+ISO were submitted to acute administration of ISO (100 mg/kg BW, s.c.), two injections separated by an interval of 24 h (that is one ISO injection between the two daily OTC treatments for the OTC+ISO group). The rats in the control group (C) have received vehicles only. Twenty-four hours after the last ISO injection, the animals were sacrificed and heart samples were collected.

Assays for MDA, AOPP and total glutathione (GSH) in the heart and standard microscopy

Tissue assays were performed according to the method described by Ohkawa et al. (1979) and as described by Taylor et al. (2015) for MDA and AOPP, respectively. The enzymatic recycling method was employed for the determination of glutathione in the heart tissue (Rahman et al., 2006). Thin longitudinal ventricular sections were made and then stained with hematoxylin and eosin (H&E) to detect leukocytes infiltration, while Masson's trichrome staining was used to spot myocardial necrosis.

* marija_bogdanovska@yahoo.com
Statistical Analysis

Groups of data were compared using one-way analysis of variance. The post-hoc test of Tukey was performed in selected instances to evaluate further differences between group pairs.

Results and discussion

ISO administration in absence of OTC treatment (ISO group) caused significant increase in the levels of heart MDA and AOPP ($p<0.01$ in both cases), and a significant decrease in the level of GSH ($p<0.05$) compared to the control rats. These changes in the oxidative stress markers may be related to the ISO-induced increased ROS generation (Garg and Khanna, 2014), accompanied by insufficient antioxidant capacity in the myocardium. Indeed, decreased GSH levels have been observed in ISO-induced rats due to the increased utilization of protective thiol containing proteins by the lipid peroxides (Nagoor Meeran and Mainzen Prince, 2011). In our study, the treatment with OTC did not increase the myocardial levels of GSH in normal rats (OTC group compared to the controls), and did not prevent the ISO-associated increase in AOPP ($p<0.001$ for C vs. OTC+ISO). However, the rats in the OTC+ISO group had cardiac GSH levels that were not significantly different compared to the controls. More importantly, the OTC treatment successfully reduced the ISO-associated increase in MDA (both comparisons “OTC+ISO vs. C” and “OTC+ISO vs. OTC” were ns). The obtained results suggest that OTC may have the ability to inhibit the deleterious effects induced by free radicals in ISO-injected rats, by means of GSH recovery that aids the maintenance of the redox balance in the cardiomyocytes (Tavares et al., 2012) and reduces the severity of the lipid peroxidation. This notion was also supported by the histological findings in the study. The myocardium of ISO-injected rats showed infarcted zone with large areas of necrosis, inflammatory cells, and separation of muscle fibers. OTC treatment reduced these histopathological changes. Additionally, the biochemical and the histopathological findings for the OTC group indicated that the OTC treatment, applied in large and frequent doses, does not possess any adverse effects.

Conclusion

This study showed that OTC mitigates ISO-induced cardiac changes. OTC treatment helps the maintenance of cardiac GSH levels, enabling the endogenous antioxidant mechanisms to cope more successfully with the ROS-induced lipid peroxidation.

References


Cardiovascular toxicity of antineoplastic medicines in Bosnia and Herzegovina

Biljana Tubić¹,²

¹Agency for medicines and medical devices of Bosnia and Herzegovina, Veljka Mladenovića bb, 78000 Banjaluka, Bosnia and Herzegovina
²Faculty of Medicine – Department of Pharmacy, University of Banjaluka, Save Mrkalja 14, 78000 Banjaluka, Republic of Srpska - Bosnia and Herzegovina

Introduction

Cancer and heart diseases are the leading causes of morbidity and mortality in many countries worldwide. Using chemotherapy and targeted therapies has led to an improvement in cancer survival rates and, unfortunately, higher cardiac adverse side effects – cardiotoxicity (Leong et al., 2019). Antineoplastic medicines have improved overall survival and progression-free survival to the oncological patients (Jemal et al., 2011; Varricchi et al., 2019). Mentioned medicines can be associated with several side effects, including cardiovascular toxicity. The National Cancer Institute defines cardiotoxicity in very general terms as “toxicity that affects the heart” (www.cancer.gov/dictionary/). Cardiotoxicity can develop in a subacute, acute, or chronic manner (Albini et al., 2018). Mitochondria are central targets for antineoplastic medicine-induced cardiovascular toxicity (Varricchi et al., 2019).

Antineoplastic-related cardiovascular toxicities have been presented in many countries especially North American and European (Leong et al., 2019). Reported results from western countries are showed that the incidence rate of cancer treatment-induced cardiotoxicity is related with several chemotherapy and targeted therapies: anthracycline (0.9%–57%), cyclophosphamide (2%–28%), trastuzumab (0%–28%) and bevacizumab (1.7%–10.9%) (Leong et al., 2019).

The Agency for medicines and medical devices of Bosnia and Herzegovina (ALMBIH) was established by the Law on Drugs and Medical Devices ("Official Gazette of B&H, No. 58/08") as an authorized body for medicines and medical devices produced and used in B&H. In 2019, ALMBIH has become full member of Uppsala Monitoring Centre – World Health Organization.

The aim of this work was to investigate the cardiovascular toxicity of antineoplastic medicines in Bosnia and Herzegovina.

Materials and methods

Data extraction and analysis

All individual case suspected reports (ICSRs) which were received by the ALMBIH for the period 2011-2019 were used as data source. The ICSRs were entered into Microsoft Excel™ tables (Microsoft Corporation, Redmond, WA, USA). The number, characteristics, and sources of ICSR, suspected drugs, adverse drug reactions (ADRs), and patient characteristics were analyzed quantitatively. The first level of Anatomical Therapeutic Chemical (ATC) classification was used to characterize...
suspected drugs in ICSR. ADRs were coded according to the Medical Dictionary for Regulatory Activities System Organ Class (SOC) classification.

**Results and discussion**

In Bosnia and Herzegovina, the highest number of received spontaneous adverse drug reactions is related with follow groups of ATC classification: group Antiinfectives for systemic use (referred as group J) (24.8%), and group Various (referred as group V) (24.8%). Behind of these groups, on the second place is group Antineoplastic and immunomodulating agents (referred as group L with 22.8%).

Based on the data from ICSRs in Bosnia and Herzegovina cardiovascular toxicities are avoided using medicines for malignancies (40%), and for cardiovascular diseases (20%). Cardiotoxicity was mainly manifested in patients aged 45-64 years (60%). It was reported equally in men and women. The most common reactions (MedDRA) were: Tachycardia (40%), Arrhythmia (20%), Bradycardia (20%) and Acute coronary syndrome (20%). The top ten suspected International Nonproprietary Names (INNs) were: Bcg vaccine (21.4%), Cytarabine (16.1%), Etoposide (10.7%), Oxaliplatin (8.9%), Rituximab (7.1%), Temozolomide (5.4%), Infliximab (5.4%), Vemurafenib (5.4%), Leuprorelin (3.6%) and Paclitaxel (3.6%).

Abnormal heart rhythm had the highest-incidence among all types of cardiovascular toxicity of antineoplastic medicines in Bosnia and Herzegovina.

Presented results are similar to previously published results (Leong et al., 2019). It is necessary to find the new biomarkers to identify patients at a high risk for the development of these complications is a high priority. Guidelines for cancer treatment that take cardiologic conditions into account are currently lacking and need to be developed (Albini et al., 2018).

**Conclusion**

Cardiovascular toxicity of antineoplastic medicines in Bosnia and Herzegovina is one of the most adverse effects arising from cancer therapeutics and a major barrier to survivorship.

**References**


Principal component analysis of sensory attributes of calcium- and magnesium enriched milk

Liljana Anastasova¹*, Tanja Petreska Ivanovska², Zoran Zhivikj², Kristina Shutevska², Rumenka Petkovska¹, Lidija Petrushevska-Tozi²

¹Institute of Applied Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia
²Institute of Applied Biochemistry, Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia

Introduction

Milk and dairy products are commonly used as vehicles for mineral enrichment because of their high nutritional value, the buffering effect on digestion and absorption processes, and the positive effects on growth (Lombardi et al., 2015). However, the addition of predefined quantity of minerals to milk can induce chemical reactions that cause changes in physico-chemical and sensory properties important for milk quality leading to reduced acceptability of such products to customers (Ocak & Rajendram, 2013).

Sensory analysis of mineral-enriched product(s) represents the ultimate method for evaluation of products quality (Schiano et al., 2017). Among the methods used, principal component analysis (PCA), a multivariate technique, is frequently applied for the investigation of the sensory attributes showing highest influence on overall acceptability of the product (Piotrowska et al., 2015).

The aim of the study was application of PCA to data obtained from sensory analysis of calcium and magnesium-enriched milk in order to investigate the most important variables affecting overall product quality.

Materials and methods

As medium for preparation of calcium- and magnesium enriched milk commercially available cow’s milk was used (1.5% fat, Alpsko, Slovenia). Accurately weighted amounts of CaCl₂ and MgCl₂ salts (Alkaloid, Skopje, Macedonia) were added in the range 0.1-0.5 g/100 g and magnesium chloride in the range 0.02-0.1 g/100 g, respectively. For the preparation, magnetic stirring was varied in the range 10-30 min. The amount of calcium and magnesium represents an additional supply to the recommended daily allowance for calcium (3.9, 19.4, and 11.7%) and for magnesium (0.8, 4.0, and 2.4%) respective to lower, upper limit and a zero level. Sensory analysis was performed by non-trained panel of 10 judges, simulating the consumers, aged 25 to 62 years. The use of non-trained personnel was due to limitations in time and financial availability, allied to the interest of simulating the behavior of a common consumer (Giune & De Lemos, 2018).

Mineral-enriched samples were placed in plastic cups for the evaluation. Water was provided for rinsing in-between the samples. Since sensory analysis immediately after production of enriched milk may not reflect actual sensory characteristics when compared to control milk, the samples were stored in refrigerator for 1 day to allow milk...
components to interact with calcium and magnesium. The milks were served 30 min after removing from refrigerator in a period of 1 week. The panelists were asked to use a 9-point scale to evaluate the taste, smell, color, consistency and the overall acceptability of the samples (0-very unpleasant, 5-neither like nor dislike, 9-very pleasant) (Kaushik et al., 2015). PCA of sensory characteristics of calcium- and magnesium-enriched milk was performed in the SIMCA 14.1 software (Umetrics, Umea, Sweden).

Results and discussion

The quality expressed by sensory attributes is an important indicator of the overall quality and health safety of milk (Piotrowska et al., 2015). In this study, all samples were characterized by adequate appearance/color during the entire examination period. However, the panelists reported significant differences between the taste and consistency of the tested milk samples enriched with the highest level of calcium chloride regardless of the level of magnesium chloride as compared to control milk. These samples showed unacceptable taste and consistency even after one day of cold storage.

PCA presents the studies variables in a reduced dimensional space. The first principal component (PC1) and the further PCs are linear combinations of the original variables which preserve maximal variance of the data. The first two PCs contain most of the variance of the data whereas the other PCs are practically unimportant (Jollife & Cadima, 2016). The PCA model of the analyzed sensory attributes of calcium and magnesium-enriched milk explained 99.5% of the variance in the data. The overall sensory quality could be described by the first two principal components, PC1 and PC2 which together explain 79.8% of data variability. Several significant correlations between the sensory attributes were found. The inspection of the loading plots of the PCA model showed that the sensory attributes smell and consistency are positioned close to each other and so are the factor taste and overall acceptability. This is in accordance with the literature finding which suggest that undesirable flavor(s)/off flavor(s)/taste in fluid milk can negatively affect milk consumption and consumer product acceptability (Yeh et al., 2017). Color was positioned by angle of 90° to the other examined sensory attributes indicating that it could not be correlated with the rest of the factors in the sensory analysis. The highest positive correlations were identified between taste and overall acceptability. Positive correlations were also found between smell and consistency and overall acceptability as well as between consistency and taste.

Conclusion

The PCA analysis of sensory attributes of calcium and magnesium-enriched milk identified taste as the most influential factor for the overall acceptability. In summary, PCA could be used as an efficient tool for identification of factors/attributes of prime importance when developing a new product in order to satisfy demanding consumers.

References


Mineral enrichment of milk – nutritional benefits and future perspectives

Liljana Anastasova¹*, Tanja Petreska Ivanovska², Zoran Zhivikj², Rumenka Petkovska¹, Lidija Petrushevska-Tozi²

¹Institute of Applied Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia
²Institute of Applied Biochemistry, Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia

Introduction

Micronutrient deficiency affects millions of people in the world in both developing and developed countries, causing considerable negative impact on individual well-being, social welfare and economic productivity (Nikooyeh & Neyestani, 2017). The proposed strategies to overcome this problem include change in the dietary lifestyle, dietary supplementation and enrichment of food (Ocak and Rajendram, 2013). Among food products that can be used as vehicles for mineral enrichment, milk and dairy products have been intensively studied. Their unique composition with the mixture of fat and aqueous phase makes it an ideal vehicle for different types of fortificants/nutrients (Nikooyeh & Neyestani, 2017). This review will provide overview of the advantages of using milk as a delivery vehicle for mineral enrichment, the most commonly added minerals for improving nutritional quality of milk and some technological aspects of the process of milk enrichment.

Milk-based micronutrient supplementation

Milk and dairy products are important source of dietary minerals, particularly calcium and magnesium, but they also contain trace amounts of iodine, selenium, iron and zinc.

Milk is the best natural source of calcium. Calcium exists in a colloidal form as caseinate-phosphate complex, readily released during digestion in vivo, hence its potential bioavailability is high. Therefore, milk has been commonly used as a vehicle for additional calcium delivery (Singh et al., 2007). Magnesium in milk and dairy products is a major contributor to dietary intake of magnesium. Milk and dairy products are, in fact, one of the main dietary sources of magnesium particularly for children, contributing approximately 10–30% of the total magnesium intake (EFSA, 2015). With better understanding of magnesium in the dairy system, there is potential for milk and dairy products to be developed to deliver increased levels of bioavailable magnesium, as well as calcium (Oh & Deeth, 2017). Milk and dairy products represent poor sources of selenium, and some studies have investigated the fortification of milk with selenium. Alzate et al. (2010) reported that fermentation of Se-enriched milk is an interesting strategy to improve human intake of several organic compound of selenium. Zinc is one of the essential minerals found in milk. Studies dealing with enrichment of milk with zinc are scarce in literature. A study of Zn enrichment at the levels of milk consumed by adolescent girls affected positively the intake and absorption of Zn. Therefore, to meet the physiological requirements and to ensure adequate Zn status, the consumption of...
enriched milk for more than 27 days can be recommended (Mendez et al., 2012). Iron deficiency comes from diverse origins but is mainly related to the low availability of iron in food products. Iron fortification of foodstuffs remains the cheapest way to avoid iron deficiency and insure the daily intake over a long period. An advantage of using milk and dairy products as a vehicle for iron fortification/enrichment is the relatively high bioavailability (Gupta et al., 2015). Iodine is naturally present in small levels in milk. However, milk is one of the largest sources of dietary iodine. There are several studies that emphasize the beneficial effect of consuming iodine-fortified milk. It seems that iodine fortified milk, in addition to iodized salt, can be considered a good dietary source to ensure iodine sufficiency especially during the lactation period (Bouga et al., 2018).

The enrichment of milk, a process where minerals in the form of salts are added to milk, is dependent on several factors such as the nutritional requirements of the intended target population, the effect of the added micronutrients on the functional or sensory characteristics of milk and the stability of the added micronutrients during processing and storage of milk (Ocak and Rajendram, 2013). The most common problem related to this process is the development of unacceptable changes in the sensorial properties such as off-flavors, bad taste as well as loss in vitamin C which is the case when iron salts are added to milk. The development of novel, advanced techniques such as micro/nanotechnology will bring revolutionary changes in the process of milk enrichment. Encapsulated ingredients have a superior performance, such as successful delivery of ingredients into foods, and a potential for enhancing bioavailability of bioactive components. Up to date, microencapsulation has been used to deliver food ingredients and bioactive compounds and can be used as a suitable mode of delivery of minerals in dairy products (Kwak et al., 2014).

**Conclusion**

Current micronutrient interventions in the field of public health aim at adding the lacking minerals to people’s diets (either by directly fortifying food or in the form of supplements). The development in modern technologies in future will improve the process of mineral enrichment resulting in “modern mineral enriched milk” in order to address consumer’s nutritional requirements.

**References**


Analysis of ergosterol as a potential contaminant in two herbal raw materials

Kristina Varsamovska1,*, Zoran Zhivikj2, Marina Topkoska1, Tatjana Kadifkova Panovska2, Lidija Petrushevskas-Tozi2, Tanja Petreska Ivanovska2

1Replek Farm, Kozle 188, 1000 Skopje, Republic of North Macedonia
2Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia

Introduction

Ergosterol is a major sterol found as a constituent of the cell wall of plants and hence can be used as a chemical marker of the presence of fungal contamination. Colonization of plants by saprophytic fungi is in increasing problem, thus the analysis of ergosterol as the most common sterol of Ascomycetaceae and Basidomycetaceae might be a reliable parameter to assess the intensity of colonization by these fungi (Lohr et al., 2017). Among other phytosterols found in cannabis plant, ergosterol was also identified and hence may be applied as an indicator of fungal contamination of raw material used to manufacture herbal medicines. In this study, two extraction procedures were compared in attempt to develop an adequate HPLC method for analyzing ergosterol in cannabis raw materials which could be applicable in routine practice.

Materials and methods

Materials

Ergosterol standard (5,7,22-ergostatrien-3β-ol; LRAB7469; purity 99.6%) was purchased from Sigma Aldrich. Two samples kindly donated from Replek Farm, Skopje were used to test the potential contamination with ergosterol: Cannabis flos, Bedrocan Nira and Cannabis flos, Bedrolite Noi IR.

Sample extraction method

The first extraction method was based on a saponification to release esterified ergosterol from cytosolic lipid particles providing total ergosterol to be quantified (Rychtera et al., 2010). Briefly, the samples were hydrolyzed with ethanolic KOH during 3 h in a water bath and afterwards the samples were vortexed with equal volume of diethyl ether. The dried extract was dissolved in a mixture of methanol:water (95:5) used as a mobile phase for HPLC analysis. The second method was simple direct extraction using hexane according to Shao et al. (2010). Multiple step extraction was performed and hexane phases were pooled and evaporated (Rotavapor, Switzerland). The extract was dissolved in ethanol before HPLC analysis.

HPLC procedure

Chromatographic system Varian 920-LC, Palo Alto, USA connected to a Discovery HS C 18 column (5 µm, 125 x 4.6 mm) heated at 30 °C and UV detector was used. Both standard and samples (20 µL) were eluted by methanol:water (95:5) at a flow rate of 1.0 mL/min. Ergosterol was detected at...
Results and discussion

Standard solutions within the concentration range of 0.5-100 µg/mL were used to perform a regression study between the observed area and the injected volume of ergosterol. The linearity and repeatability were assessed with the standard solutions which have been injected six folds successively. Recovery was assessed on the samples (ether and hexane extracts) (n=3) spiked with standard solution of ergosterol. Better recovery and separation of the analyte ergosterol was obtained after alkaline hydrolysis of the samples combined with subsequent extraction with ether. Namely, the chromatograms of the samples extracted by this procedure and loaded with ergosterol at a known concentration showed no interfering peak near the retention time of ergosterol. In contrast, direct extraction with hexane and with added known concentration of ergosterol, showed a peak which was not clearly separated from ergosterol.

Chromatographic analysis of the alkali-hydrolyzed samples in which prior to extraction was added a standard solution of ergosterol with the lowest concentration (0.5 µg/mL), showed the adequacy of the method for the identification and quantification of ergosterol which concentration in a given sample is not less than 0.5 µg/mL. Considering the limit of detection and quantification of ergosterol, the applied method is characterized by significant analytical potential for the qualitative and quantitative determination of low levels of ergosterol in different cannabis raw materials. Ergosterol concentration in grains is highly correlated to the production of fungal toxins (Perkowski et al., 2008). Improper conditions of cultivation or storage of cannabis may also result in ergosterol production. McKernan et al. (2015) and Verweij et al. (2000) have detected significant contamination of cannabis with several toxicogenic fungi from the genera *Penicillium* and *Aspergillus*. Hence, the method described in this study may be applicable due to the easy, fast and efficient extraction of ergosterol from tested samples allowed for precision and repeatability of the subsequent HPLC analysis. The method is simple, sensitive and relatively inexpensive and can be adapted for routine use in specialized laboratories which control the quality of cannabis raw materials for the production of herbal medicinal products.

Conclusion

The use of ergosterol as an analytical parameter for assessment of the potential fungal contamination of raw cannabis can help adequate preventive measures to be developed and on time control strategies to be applied by the manufacturers.

Acknowledgements

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References


Vibrational spectroscopy studies on biosynthesized silver nanoparticles

Darinka Gjorgieva Ackova¹*, Katarina Smilkov¹, Aleksandar Cvetkovski¹, Petre Makreski²

¹Department of Pharmacy, Faculty of Medical Sciences, Goce Delcev University, 2000 Stip, North Macedonia
²Institute of Chemistry, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University, 1000 Skopje, North Macedonia

Introduction

Silver nanoparticles (AgNPs) have been intensively investigated due to great potential applications, for instance, in technology, physical, biomedical and pharmaceutical sciences. These nanoparticles can be synthesized by chemical, physical and biological methods. In general, physico-chemical methods can be toxic, expensive, produce NPs with low yield and limitations for medical/pharmaceutical use (contamination from precursors, solvents, etc.). Thus, the biosynthesis of NPs using plant extracts has become very important topic for research in recent years (Adil et al., 2015; Agressott et al., 2020). Plant extracts contain variety of natural compounds with different molecular structures that exhibit reduction to metal ions to elemental metal nanoparticles. Hence, preparing metal nanoparticles with plant extracts match the concept of green chemistry for their production and also increased biocompatibility as great advantage in their application.

The increasing use of nanomaterials in wide range of products has initiated concern on their interactions with biological systems and potential cause of toxicological effects. Already some of the potential risks of nanotechnological products has been described, but their general toxicity remains largely unknown due to the lack of scientific data about what happens when NPs entering into living cells (Huang et al., 2017; López-Lorente and Mizaikoff, 2016).

As information about the physical, chemical, and biological properties of the NPs is still missing, in this work, we try to go a step further in revealing characteristics of biosynthesized AgNPs by performing a vibrational spectroscopic investigation.

Materials and methods

NPs are obtained by using AgNO₃ as a silver precursor and the aqueous plant extract as a bioreducing agent. The overall incubation process was carried out on room temperature, using orbital shaker in dark conditions, and the obtained AgNPs were purified through centrifugation.

Results and discussion

NPs with inherent infrared absorptions or functional groups present at their surface, and also different ligands attached to them, may be identified according to their vibrational signatures in a rapid, precise, and non-destructive way by directly characterization via Raman spectroscopy. Furthermore, vibrational spectroscopy allows the
detection of functional groups and adsorbed molecules at the surface of NPs, as well as monitoring changes of the interactions with other molecules including biological systems (Lopez-Lorente, 2016; Rygula et al., 2013). Raman spectrum of AgNPs was recorded in the region 2000–200 cm\(^{-1}\). The peaks at around 500, 900-1000, and 1500-1600 cm\(^{-1}\) might be assigned to a shift of the twisting, rocking, and scissoring modes of NH\(_2\) group, respectively, indicating an amine-silver interaction (Liu-Bin et al., 2011). Symmetric and asymmetric C=O stretching vibrations of carboxylate group, corresponds to bands registered at region 1300-1600 cm\(^{-1}\). Also, stabilization of AgNPs by chemical bonding of the amino and/or carboxylate groups of the plant molecules with the silver atom can be detected. Our future analysis will be focused to investigate possible interactions occurring, which can help to predict potential toxicity and identify agent-induced alterations occurring within cells.

**Conclusion**

It is expected that one combined/integrated analytical techniques approach will provide next-generation tools for studying nanoparticles in a wide variety of complex application in pharmaceutical or biomedical scenarios.

**References**


Critical points in Comet assay silver staining procedure

Misko Milev, Viktorija Maksimova, Milkica Janeva, Tatjana Ruskovska*

Faculty of Medical Sciences, Krste Misirkov 10-A, 2000 Stip, North Macedonia

Introduction

Comet assay is one of the most common methods for measuring DNA damage in eukaryotic cells. In 1984, Ostling and Johanson demonstrated movement of DNA strands when nuclei were exposed to an electric field. Later, Singh et al. (1998) modified and optimized the method using alkaline conditions during electrophoresis, thus increasing specificity and reproducibility of the method. In the recent years, this assay has gained in popularity because of its simple, economical, versatile and sensitive procedure. This assay requires only a small number of cells per sample and provides collection of data at individual cell level, allowing robust statistical analysis. According to Azqueta and Collins (2013), there is a number of comet assay variations, applicable to different types of samples, such as: peripheral blood cells, cell lines, buccal mucosa, yeasts, cancer cells and plant cells.

This method can measure single-strand or double-strand DNA breaks, DNA cross-links, alkali labile sites, base/base-pair damages and apoptotic nuclei (Collins, 2004). It has widespread applications in the area of testing novel pharmaceuticals for genotoxicity, monitoring environmental contamination with genotoxins, human biomonitoring and molecular epidemiology, diagnosis of genetic disorders and fundamental research in DNA damage and repair. During electrophoresis the damaged cells acquire the shape of a comet, hence the cells are called comets and the procedure comet assay. These comets can be visualized using fluorescent staining method or silver staining method.

In this communication, we have considered silver stained comet assay images because they are preferred in clinical applications (Jackson and Bartek, 2009). This is mainly because fluorescent staining requires high quality fluorescence microscope. Further, with fluorescent staining the slides cannot be stored for a long period of time and they should be photographed and analyzed immediately. The advantages of silver staining method are that it is inexpensive, slides can be preserved for a long time, and the analysis can be carried out using a simple light microscope. The main disadvantage of silver staining compared to fluorescent images is that silver stained images have a high level of background noise, which should be diminished as much as possible. Therefore, we have identified the critical points of comet assay silver staining procedure, as established and optimized in our laboratory.

Materials and methods

Peripheral blood mononuclear cells were embedded in 0.7% LMA (low melting agarose) on a microscope slide (around 10^6 cells per slide) precoated with 1% NMA (normal melting agarose). Then cells were lysed at 4 °C for minimum of 1 hour. Lysis solution was prepared with NaCl (2.5 M), EDTA (100 mM) and Tris base (10 mM), pH=10. Before use, cold 1% Triton X was added. This procedure disrupts the membranes and removes cytoplasm and histones.

* tatjana.ruskovska@ugd.edu.mk
The remained coiled DNA, so called nucleoid, was incubated at 4 °C for 40 min at pH > 13 in the electrophoretic buffer (300 mM NaOH, 1 mM EDTA), thus allowing for DNA to unwind. A 300 mA current with voltage of 0.8 V/cm across the field was applied for 30 minutes, which allowed the damaged DNA parts to travel toward the anode.

After electrophoresis, slides were flooded 3 times for 5 minutes with neutralizing buffer (pH=7.4) and then with deionized water, after which the sides were allowed to dry for 1 hour at room temperature.

Then the slides were fixed for 10 minutes in fixative solution (1.5% w/v trichloroacetic acid, 5% w/v zinc sulfate and 5% glycerol). After fixation the slides were washed 3 times with deionized water and left overnight to dry at room temperature. Before silver staining slides were re-hydrated for 5 minutes in deionized water.

The silver staining solution was prepared fresh in following sequence: 34 mL of solution B (5% w/v sodium carbonate, 0.2% w/v ammonium nitrate, 0.2% w/v silver nitrate, 0.5% w/v tungstosilicic acid, 0.15% v/v formaldehyde) to 66 mL of Solution A (5% w/v sodium carbonate). After staining of the slides, they were washed 3 times with deionized water. The staining was stopped with a treatment of slides for 5 minutes at 1% acetic acid solution.

Results and discussion

As an alternative method for dyeing, the silver staining is more economical but requires much more time and experience. The optimization of silver staining method in single cell electrophoresis has shown many critical issues which should be strictly considered when applying this type of staining to the examined DNA:
1. High cleanliness of laboratory glass – all the glass material used, should be pretreated with 50% of nitric acid solution, then washed with detergents and deionized water.
2. Microscopic slides should be dried overnight at room temperature after coating them with NMA.
3. Time of silver staining – three series of 10 minutes with fresh solution should be performed on low intensity shaker until light gray color is formed. Slide orientation and position should be modified because waves in the staining solution can cause aggregations of silver particles.
4. Staining tray should be covered with aluminum foil to provide dark conditions.
5. Freshness of staining solution is another sensitive variable. The staining solutions should always be freshly prepared with intense mixing and with minimum exposure to intense light.

Other parameters, not specific to silver staining, should be also taken in consideration. Concentration of LMA, cell density, time of unwinding of DNA in high pH solution, voltage, time and temperature during electrophoresis can significantly influence the outcome of the assay, so they should remain constant.

Conclusion

Taking into account all these critical points, silver staining can produce excellent quantification of DNA damage with comet assay using only standard light microscopy.

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References

Dietetic approach in patient with type 2 diabetes mellitus - a case report

Bojana Janeku*, Dafina Boshkoska, Elena Karabeleski, Menka Andreska, Suzana Atanasovikj, Dragana Mladenovska, Kristina Mladenovska, Lidija Petrushevska-Tozi, Tanja Petreska Ivanovska, Aleksandra Kapedanovska Nestorovska

Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, N. Macedonia

Introduction

The dietetics as a discipline and the dietitians as health professionals integrate principles of the health, biological, physiological, behavioral, social and food and nutrition sciences with management and communication to provide and maintain optimal human health within flexible scope of practice. The main activities include adequate screening/assessment of patients’ needs for nutritional therapy (NT), detecting the presence or risks for developing a nutrition-related problem, development of nutritional diagnosis, nutrition intervention and monitoring and evaluation of the nutrition care plan. Within that, a diabetes dietitian can offer specialist evidence-based dietary advice to patients with diabetes while considering factors including nutritional status, medication, diabetes control and lifestyle (Evert et al., 2019; Roth, 2010).

Type 2 diabetes mellitus (T2DM) is a chronic, metabolic disease with insulin resistant body cells. Risk for developing ketoacidosis is low, but elevated levels of blood glucose lead, over time, to serious damage to many systems. Although T2DM may experience marked hyperglycemia, many patients do not require insulin injections and diabetes can be controlled with pharmacotherapy (PT) i.e. oral hypoglycemic agents and proper lifestyle that includes a diet/NT and regular physical activity. In this paper, a patient case is presented to show that the synergistic effect of PT and NT helps in control of T2DM and reducing co-morbidities. Also, the role of dietitian in providing the best diabetes control possible through a good understanding of the condition and the best use of medications is emphasized.

Case presentation

A 55-years old female patient (33.2 BMI) with a (family history of) T2DM diagnosed 30 years ago was hospitalized for foot ulcer, which was edematous, necrotic, with exudates and hot. Patient’s medical history included hypertension and lipid profile that promoted cardiovascular complications. Microalbuminuria and disorders in electrolyte status indicated nephropathy and chronic kidney disease (CKD) in development. Symmetric distal peripheral neuropathy was also evidenced. In addition, menopausal symptoms and changes were experienced (e.g., weight gain) for which hormone replacement therapy and vitamins and minerals were prescribed. The patient had a poor lifestyle (pasta-rich diet, cigarettes and alcohol consumption and no physical activity). Laboratory tests showed increased values of following parameters: glucose 178 mg/dL, HbA1C 8.9%, HDL-C 48 mg/dL, triglycerides 180 mg/dL, creatinine 2.5 mg/dL, blood urea nitrogen 37
mg/dL, potassium 6.1 mg/dL, microalbumin 45 mg. In addition to regular therapy (metformin á 500 mg, twice a day and amiloride hydrochloride á 5 mg/hydrochlorothiazide á 100 mg, propranolol á 80 mg (prolonged release) and atorvastatin á 10 mg per day), amoxicillin/clavulanate 1000 mg twice a day was administered for treatment of diabetic foot ulcer.

Discussion

T2DM is a complex disorder that is heterogeneous in nature, so NT plan should be implemented by prioritizing metabolic problems. In T2DM patients receiving PT, energy needs are calculated individually based on the glycemic index. The most acceptable method of NT is to count carbohydrate units in each meal.

Nutritional assessment of the patient, according to the BMI and the information on the significant increase of the body weight over the past period, pointed to obesity class 1. Mayor problems in NT were allocated in poorly controlled T2DM that correlated with inappropriate lifestyle and insufficient awareness of self-control and contributed to development of long-term cardiovascular and nephrological complications. With nutritional diagnosis, inadequate ratio of major nutrition components and low intake of dietary fibers was detected. In addition, potential interactions between alcohol consumption and metformin were identified as a risk for developing lactic acidosis. Therefore, nutritional intervention was focused on changing nutritional habits, controlling and maintaining a balanced diet and mandatory lifestyle changes in terms of physical activity and cessation of alcohol and cigarette consumption. The priority was to achieve optimal glycemic control (by reducing HbA1C for 1-2%), control of lipid status (by reducing blood pressure and preventing further cardiovascular deterioration) and consequently, to control the nephropathy and peripheral vascular disease (hygiene treatment and exercise), all through joint venture of PT and NT. Extra goal was to reduce body mass by 10-15% and to improve insulin sensitivity. According to the total energy expenditure calculation, the patient received energy intake of up to 2000 kcal/day, with the distribution of macro-nutrients according to her diagnosis as follows: 50-60% carbohydrates (with a low glycemic index); up to 10% protein; up to 30% fat. Since the patient was on a normal diet (no need for liquid food), the design of meals included up to 1200 kcal from carbohydrates (60-75 g in main meals, max. 200-225 g/day), up to 200 kcal from protein, and up to 600 kcal from fat. Since the patient had bad lifestyle and poor self-control, after the hospital stay, she was trained in proper food and nutrition management, better understanding of the complications and their management and was advised to perform regular controls. She was offered self-monitoring guidelines and an appropriate psychological support for managing the stress. In addition, she was educated for proper use of food and drugs in order to minimize the interactions. Since metformin reduces the absorption of vitamin B_{12} and folates and increases the risk of anemia, the diet rich in vitamin B was recommended. To avoid potassium rich food was also recommended because of potassium-spearing diuretic in therapy. Despite debridement and therapy of leg ulcer, it took 4 weeks to fully recover (with reduced calorie intake and extra PT). Physical activity was introduced afterwards, gradually, with suggested menus from 2000 to 2500 kcal and mandatory glucose monitoring by scheme.

Conclusion

T2DM treatment comprising NT, PT and physical activity may provide adequate glycemic control. Complete control of diabetes requires awareness of the importance of each of the therapies, the synergistic relationship between them and the maximum adherence to each therapy, including nutrition interventions by the certified dietitian.

References


Dietetic approach in patients with irritable bowel syndrome – a case report

Menka Andreska*, Bojana Janeku, Dafina Boshkoska, Elena Karabeleski, Suzana Atanasovikj, Dragana Mladenovska, Aleksandra Kapedanovska Nestorovska, Tanja Petreska Ivanovska, Kristina Mladenovska, Lidija Petrushevska-Tozi

Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa No. 47, 1000 Skopje, N. Macedonia

Introduction

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder, with high prevalence of 7-21% globally. One of 10 people has IBS or some of the symptoms due to various factors. Different countries have different incidence rate - the average of the available statistics is 2-70/1000 patients per year. It is a syndrome with a set of symptoms that can occur in different forms, intervals and intensity. IBS is a lifelong problem with a major impact on the quality of life of the patient, family and environment. Occurs more often in the female then male (2 of 3 cases are female). In terms of age, it usually occurs before the age of 50 (between 20-30 years), but there is no age limit as symptoms can be also encountered in children (McKenzie et al., 2016).

Symptoms of IBS have been described in medicine since 1849, using various names such as spastic (irritable) bowel syndrome or neurogenic mucous colitis. Today, this syndrome is defined as “intermittent abdominal pain or discomfort for 4 days in the last 2 months, in the absence of organic disease, followed by one or more of the following changes: changes in defecation rate, frequency, shape, and/or stool appearance, bloating, cramps and gas” (Nelms, 2010). Because of the nature of the syndrome, nutrition care plan should be provided to ensure better quality of life as well as favorable clinical outcome.

In this paper, a case of patient with IBS is presented and the role of an accredited dietitian in providing, expert dietary advice for management of IBS symptoms and nutritional needs is emphasized.

Case presentation

A 33-year-old woman was diagnosed with IBS. She had intermittent episodes of diarrhea and constipation and complains of distension and abdominal pain. Her physician advised her to supplement her diet with fiber and psyllium. The patient did not want to replace white bread with whole wheat bread. She also refused to use fiber (psyllium) in the form of a dietary supplement because she was convinced that the fibers helped with constipation but worsened diarrhea. She considered adding yogurt to her diet to see if it would help. For breakfast, she usually took a glass of orange juice, two slices of white bread with peanut butter and coffee, for snack small portion of chips, for lunch hamburger and a diet coke, for afternoon snack a portion of crackers with cheese and a glass of white wine. The usual dinner consisted of chicken meat prepared in sauce with

* m.andreska@yahoo.com
potatoes, broccoli, as dessert ice cream and coffee and before bedtime, milk and cookies or apples. Given the health problems associated with the intestines, the patient had symptoms of depression. Physician recommended pharmaco-therapy that included oral magnesium hydroxide, linaclotide or loperamide as needed, dicyclomine as needed, fluoxetine at recommended dose, and gabapentin as needed.

Discussion

Nutritional intervention in patients with IBS should be based on the current situation. General recommendations include taking small meals throughout the day as well as probiotics to regulate the intestinal flora and reduce bloating and gas. To detect foods that exacerbate symptoms it is recommended that the patient keep a diet diary. In the phase of predominant diarrhea, one recommends adequate fluid intake, primarily water and herbal tea, restriction of caffeine-containing beverages, restriction of foods containing insoluble fiber (whole grain bread, nuts, seeds), restriction of eating fresh and dried fruit to 3 servings per day, reduction of starch intake and sugar free foods (containing sorbitol and xylitol), consumption of probiotic-containing products (yogurt, fermented dairy products) and avoiding fat-containing foods (chips, burgers, fried foods and sweets) as they can worsen diarrhea. In the predominant constipation phase, dietary fiber intake is recommended. It improves constipation, but causes an increased amount of gas in the intestine, which causes pain, flatulence and cramps. Individual customization of the selection of cereals is also recommended and of the quantity that will suit personally. Intake of fiber rich foods should be gradual, because any sudden increase in fiber intake can worsen symptoms. Symptoms of constipation can be improved by combination of whole grains with fruits and vegetables. Oatmeal and linseed are good sources of soluble fiber, which help to soften the stool and ease the passage and can also help alleviate the symptoms of gas and bloating. Adequate fluid intake of at least 8 glasses of caffeine-free fluids per day (best water) has to be ensured.

Since the patient refused to replace the food that she was usually consuming, it was important to receive timely and accurate nutrition information in order to adopt and implement a diet plan and condition control. The patient was educated about the composition of the food and its benefits and the complications that it can make in its condition. Due to inadequate intake and selection of foods and liquids according to the patient’s condition there was a risk of malnutrition. Thus, in the acute phase of the condition, the patient was advised to take an enteral nutrition that will help relieve the symptoms faster and achieve remission.

Considering that the condition significantly affected her mental state, consultation with a psychologist was also recommended in order to obtain a complete treatment that will improve the overall condition.

Conclusion

Dietary changes in patients with IBS syndrome should be implemented in order to alleviate symptoms and improve quality of life. Monitoring patient’s progress is essential and a health professional with an expertise in dietetics can significantly help in dietary management, education and counseling of patients.

References


Nutrition in COPD patients - case study

Elena Karabeleski*, Lidija Petrushevska-Tozi, Bojana Janeku, Suzana Atanasovikj, Dafina Boshkoska, Menka Andreska, Dragana Mladenovska, Tanja Petreska Ivanovska, Aleksandra Kapedanovska Nestorovska, Kristina Mladenovska

Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa No. 47, 1000 Skopje, N. Macedonia

Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive disease that limits airflow through either inflammation of the lining of the bronchial tubes (bronchitis) or destruction of alveoli (emphysema). COPD according to statistics from the WHO is the fourth leading cause of death overall in the face of decades and expected to be increased.

Medical treatment for individuals with COPD involves lifestyle changes, including smoking cessation, avoiding smoke and other air pollutants, exercising as tolerated and good nutrition. As COPD progresses, symptoms such as shortness of breath, taste alterations due to dry mouth, fatigue, early feelings of fullness, etc. can contribute to decreased food intake. Disease-related malnutrition is a common problem in individuals with COPD, with between 30% and 60% of inpatients and 10% and 45% of outpatients said to be at risk (Collins et al., 2013). A prolonged decrease in food intake can lead to significant weight loss and malnutrition. For better clinical outcome and patients’ quality of life, the nutrition care plan should be provided by dietitian.

The aim of this paper is to present an approach of nutritional therapy in a patient with COPD and to emphasize the role of the certified nutrition in nutrition assessment, diagnosis and intervention in COPD patients.

Case presentation

In a 62-year-old female patient with a medical history of bronchitis and upper respiratory infections, COPD was diagnosed five years ago. The patient was admitted to the hospital complaining of severe dyspnea and fatigue, showing signs of cyanosis, and using accessory muscles for expiration. PEFR of 190 L/min and pulse of 100/min were determined. The patient was not a smoker (she quit smoking 5 years ago). She was additionally diagnosed with acute exacerbation of COPD, secondary to pneumonia. The patient indicated that her appetite was poor and the food did not taste good. Her highest adult weight was 63-68 kg (about 5 years ago), and 54 kg at the time of hospitalization (BMI 27.6 kg/m²). She complained that her dentures were fitting very loosely. She had 1+ bilateral pitting edema, decreased breathing sounds and prolonged expiration with wheezing and she used accessory muscles at rest. Laboratory analysis pointed to albumin 3.4 g/dL, pH 7.29, PaCO₂ 50.9 mmHg, PaO₂ 77.7 mmHg, O₂Sat 92%, HCO₃ 29.6 mEq/L. Her usual dietary intake included: coffee, juice and dry cereal, with a small amount of milk in the morning and one other larger meal during the day, usually at lunch (consisted of meat, vegetables, rice, potatoes or pasta). She admitted that she was eating only small portions. At night she often had a bowl of soup. She used to drink cola throughout the day (usually 1 L). Her pharmacotherapy was consisted of inhaler salbutamol 2.5 mg and ipratropium bromide 500 mcg and i.v cefuroxime 750 mg, each three
times daily, oral aminophylline retard 225 mg two
times daily and oral prednisolone in the morning in
dosage regimen adjusted by scheme until achieving
the desired clinical response.

Discussion

For nutrition assessment, patient’s biochemical
data and anthropometrical measurements were
analyzed, while for nutrition diagnosis to be
established, problem, etiology and signs/symptoms
(PES I) of the patient, inadequate calorie intake (due
to loss of appetite, taste deterioration and problems
with dental prostheses) (PES II) as well as
unintentional weight loss due to COPD (reduction of
10 kg compared to normal weight) (PES III) were
considered. By MUST screening tool, score ≥ 2 was
determined and high risk for malnutrition, even with
BMI 27.6 kg/m^2.

Nutrition intervention included recommendations for intake of small frequent meals
(5-6 meals per day), dense in nutrient content, with
sufficient calories for weight gain, including meals
that require little preparation (e.g. liquid nutritional
supplements), resting before meals and taking daily
dose of multivitamins (vitamin C, D, E). The patient
was advised to replace the use of carbonated soft
drink with water and naturally squeezed juice.
Consuming juice during a meal was not
recommended, avoiding was suggested, as the
patient was quickly sated. The benefits of fruits and
vegetable in chronic and acute respiratory conditions
were emphasized due to the antioxidants, minerals,
vitamins, flavonoids, phytochemicals, and fiber
contents. Omega-3 polyunsaturated fatty acids
(PUFA) were also included due to their anti-
inflammatory effect and potential benefit in COPD
and also in malnourished patients. Oral nutritional
supplements (standard formula 300 kcal) were also
introduced as a replacement for one meal 1-2 times a
day. Based on Harris Benedict formula, total daily
calorie needs for the patient were calculated, being
1350–1620 kcal (140% above BMR), or 25-30
kcal/kg, with 20% of total intake for protein (1.2-1.7
g/kg) (Deutz et al., 2014).

The use of glucocorticosteroids in the treatment
of COPD has been shown to increase the incidence
of osteoporosis. Glucocorticosteroids decrease
the intestinal absorption of calcium and increase urinary
excretion, resulting in an increase in parathyroid
hormone levels and bone resorption. Therefore,
measurement of bone mineral density was indicated,
including daily intake of calcium 1200 to 1500
mg/day and 400 IU of vitamin D at least (Bergman
and Hawk, 2010). Nutrition monitoring with 24-hour
dietary recall method was recommended, including
monitoring of the ability to perform every day
activities, physical appearance, and appetite, disease
progression and also weight change. Weight gain of
2 kg in period of > 2 weeks was used as a
therapeutic target associated with functional
improvements (Collins et al., 2013).

Conclusion

Malnutrition is prevalent among patients with
COPD and can have serious consequences for both
the individual and local health economy. The causes
of malnutrition in COPD are multifactorial and
include reduced energy intake due to decreased
appetite, depression, lower physical activity and
dyspnea while eating. To achieve a more effective
nutrition intervention, systematic nutrition
management, continuous data reporting, and the use
of an interdisciplinairy team approach (including
certified dietitian) are needed.

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Dietetic approach in patient with dehydration and electrolyte imbalance caused by diarrhea - a case report

Dragana Mladenovska*, Dafina Boshkoska, Elena Karabeleski, Menka Andreska, Suzana Atanasovikj, Bojana Janeku, Aleksandra Kapedanovska Nestorovska, Tanja Petreska Ivanovska, Kristina Mladenovska, Lidija Petrushevska-Tozi

* mladenovska.dragana@gmail.com

Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa No. 47, 1000, Skopje, N. Macedonia

Introduction

Acute diarrhea is defined as three or more episodes of partially formed or watery stool per day, which lasts less than 14 days. Diarrheal illness accounts for approximately 2.5 million deaths per year worldwide (de Bruyn., 2010). Bacterial and viral infections account for most episodes of acute diarrhea in adults, with viruses being the most common infectious cause in the community. Electrolyte imbalance as a result of acute diarrhea is common and frequently unrecognized resulting in morbidity and mortality. Therefore, effective replacement of electrolytes in diarrheal patients is necessary based on exact knowledge of changes in the composition of body fluids. Early recognition of the acute diarrhea and analysis of common electrolyte abnormalities is necessary to provide correction. The first line of treatment should include solutions for oral rehydration and adequate nutritional support that will prevent electrolyte imbalance from progressing (Casburn-Jones and Farthing, 2004). Based on a patient case, this paper aims to stress the importance of nutritional support and the role of certified dietitian in preventing severe consequences from acute diarrhea.

Case presentation

Eighteen-year-old male, recently returned from a two-week trip to Middle East country was feeling ill for several days, which started the day before arriving home. He reported 5 to 10 episodes of diarrhea each day, which was resolved after taking loperamide. Fecal smear indicated gross blood with leukocytes. Admitting diagnosis was moderate dehydration with R/O bacterial vs. viral gastroenteritis.

After physical assessment, the following parameters were evidenced: general appearance- lethargic 18-year-old male; vitals- temperature 38.6 °C; blood pressure 80/65; heart rate 89 BPM; respiratory rate 22 BPM; heart- moderately elevated pulse; eyes- sunken, sclera clear without evidence of tears; ears- clear; nose- dry mucous membranes; throat- dry mucous membranes, no inflammation; genitalia- unremarkable; neurologic- alert, oriented, irritable; extremities- no joint deformity or muscle tenderness, no edema; skin- warm, dry; reduced capillary refill (approximately 2 seconds); chest/lungs- clear to auscultation and percussion; abdomen- tender, nondistended, minimal bowel sounds.

After nutritional assessment, the following anthropometric measurements were reported: height- 188 cm; weight 81 kg; BMI 22.9 kg/m². After
biochemical testing the following data were obtained: total protein 7.2 g/dL, albumin 4.9 g/dL, sodium 154 mmol/L, potassium 3.2 mmol/L, chlorides 107 mmol/L, phosphates 4.0 mmol/L, BUN 21 mg/dL, chromium 1.4 mg/dL, Hgb 15.5 g/dL, Hct 41%, WBC 17×10³/mm³.

Discussion

The goals of the nutritional intervention in acute diarrhea are to restore lost fluids, normalize water, electrolytes and acid-base balance, reduction of gastrointestinal motility and initiation of normal stool formation. Re-colonization of the gastrointestinal tract should be also established. With a proper diet regimen, the patient returns to a normal diet without worsening symptoms (Nelms, 2010).

Based on the patient data, nutritional diagnosis included: (P) inadequate fluid and food intake related to (E) increased losses of fluids and fever as evidenced by (S) diarrhea, hypernatremia, and elevated serum chloride ions.

The patient had confirmed mild dehydration and the first step of nutritional intervention was to administer oral rehydration therapy. The fluid requirements were calculated based on the body weight, being 2835-3240 mL (30-35 ml/kg × 81 kg). For the best outcome, a low osmolality oral rehydration solution (ORS) (240-250 mOsm/L) was prescribed, knowing that high osmolality ORS cause additional water absorption in the gastrointestinal tract and may worsen the condition and diarrhea.

Nutrition therapy was designed to prevent the consumption of beverages containing large amounts of simple carbohydrates (lactose, sucrose, and fructose), sugar alcohols (sorbitol, xylitol, and mannitol), caffeine and non-alcoholic foods fibers (cellulose). The patient was advised to consume foods containing soluble fiber and pectin (to promote stool formation). As best starters, green bananas that contain a lot of pectin, toast with white bread or boiled rice were recommended. Other foods were also slowly introduced to provide a sufficient variety of nutrients. The patient was advised not to consume spicy and hot foods as well as sodas because they can cause additional irritation in the gastrointestinal tract (GIT).

Another mandatory step in the treatment of diarrhea is administration of probiotics and prebiotics in order to re-colonize GIT and restore normal function and nutrient absorption. Foods that naturally contain prebiotics and probiotics were recommended to be consumed more often.

Daily menus were created and at the beginning of therapy meals were small but frequent, usually every 3-4 hours, with several interruptions when the diarrhea returned, to determine which of the foods worsened the condition. The patient was advised to drink minimum of 2.8 L of water during the day (water, unsweetened tea or rehydration drinks; the amount of yogurt was counted in daily fluids).

To evaluate the outcome of the nutrition therapy, the following parameters were regularly monitored and recorded: serum electrolytes, weight, food and fluid intake, food tolerance, frequency and amount of diarrhea.

Conclusion

Adequate nutritional support plays critical role in restoring normal function of GIT and preventing future complications of acute diarrhea. For this to be achieved, the diarrhea dietitian can help discover how different foods impact the patient’s digestion and what triggers the symptoms. He/she can point to the adequate food items and solutions for proper hydration; food supplements for restoring balance in the GIT and in addition, helps in modifying lifestyle habits.

References

Nutritional management of chronic kidney disease – a case report

Dafina Boshkoska1*, Bojana Janeku1, Elena Karabeleski1, Menka Andreska1, Suzana Atanasovikj1, Dragana Mladenovska1, Jasminka Patcheva2, Kristina Mladenovska1, Lidija Petrushevsk-Tozi1, Tanja Petreska Ivanovska1, Aleksandra Kapadanovska Nestorovska1

1Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, N. Macedonia
2Pharmaceutical Chamber of Macedonia, 50th Division 34, 1000 Skopje, N. Macedonia

Introduction

Chronic kidney disease (CKD) is typically a progressive syndrome in which kidneys lose their ability to filter blood, concentrate urine, excrete waste and maintain electrolyte balance. CKD is an important public health issue that consumes major global health care resources. The worldwide prevalence of renal dysfunction is estimated to be between 11% and 13% (Hill et al., 2016).

Dietetic-nutritional therapy (DNT) is an important component of conservative treatment of patients suffering from CKD, aiming to maintain an optimal nutritional status, prevent and/or correct signs, symptoms and complications of CKD and to delay starting of dialysis. Modulation of protein intake, adequacy of caloric intake, and control of sodium and potassium levels as well as reduction of phosphorus intake is the essential points of DNT (Cupisti et al., 2018).

In this paper, a case of patient with CKD, stadium 5 is presented, with an aim to emphasize the importance of setting nutritive diagnosis in these patients and establishing a plan for nutritive intervention. Criteria for nutritive monitoring and evaluation of the outcomes associated with each recommended nutritive intervention are also given.

Case presentation

A 26-years old, female patient (77 kg, 152 cm height, BMI 33.33 kg/m²) was hospitalized at the Clinic of Nephrology, with symptoms of anorexia, edema, and difficulties in breathing, pruritus and inability to urinate. Previous medical history included renal insufficiency, hypertension and diabetes mellitus type 2. The patient was diagnosed with CKD, stadium 5 and was about to start hemodialysis. CKD was caused by untreated diabetes mellitus and was presented by damages of glomerular wall, increased perfusion, protein loss, edema and metabolic acidosis, uremia, azotemia, hyperphosphatemia, hyperkalemia, oliguria and hypertension.

The patient had poor appetite, but had 4 kg recent weight gain. In the past 24 hours, she had toast and cola for breakfast, ham and cheese sandwich and ice tea for lunch and spaghetti portion and ice tea for dinner.
Actual pharmacotherapy included captopril, calcitriol, erythropoietin, vitamin and mineral supplements and glucophage.

Discussion

The nutritive intervention in this patient should include prevention of malnutrition, minimization of uremia, decreasing of complications (cardiovascular diseases, anemia and secondary hyperparathyroidism), as well as normalization of blood pressure. In addition, in patients with CKD (stadium 4-5), a diet with non-monitored intake of energy, protein, sodium and phosphate hastens and exacerbate clinical and metabolic alterations related to advanced CKD. This can reduce the effectiveness of drug therapy or require an increase in dosage, while DNT must be managed according to the stages and criteria of any other drug therapy (Cupisti et al., 2018). Therefore, the patient’s diet should be rich in proteins and the intake of potassium, sodium, phosphates and water should be strictly regulated. Consumption of food with appropriate energy value is very important for balancing the optimal nutritive status. Fat intake should be also controlled. If the patient is on dialysis, foods rich in potassium, sodium and water should be limited. There should be restriction on consumption of fruits, vegetables, nuts and seeds, chocolates and milk products.

Hence, the recommendations for DNT in the patient include: consumption of food poor in potassium (apple, apple juice, canned apricots, berries, grapefruit, tangerine, peaches, pears, pineapple, plums, watermelon (limit on 1 piece), asparagus, beans, cabbage, carrots (boiled), cauliflower, celery, cucumber, eggplant, mushrooms, onion, parsley, paprika, rice, bread, sweets without nuts and chocolate, potassium from the fresh vegetables is removed by blanching); food poor in sodium (fresh and frosty meat, eggs, milk products (only once a day), fresh and frozen vegetables without added salt, vegetable olive and canola oil, garlic, onion, black pepper, lemon, homemade soups and canned food without added salt); decreased intake of liquids; intake of less salted food; administration of medicines with the liquids from the meal; taking liquids only when thirsty (for moisturizing the mouth, bonbons without sugar to be used, ice cream, sorbet and yogurt are considered liquids); avoiding food rich in phosphorus (chocolate, beer, cocoa, cola drinks, cheese, milk, yogurt, ice cream, liver, sardines, bean, pea, soya, lentils, wholegrain cereals and nuts).

As an example, the daily menu of the patient could include: ½ glass apple juice, 100 ml full fat milk, 1 muffin, 220 ml filter café and rice cereals for breakfast; turkey sandwich (56 g meat, 2 slices white bread, 1 tablespoon mayonnaise, 2 leaves lettuce), ½ glass pomegranate juice, ½ glass canned peaches and 2 small graham crackers for lunch; and 56 g roasted pork, ½ glass white rice, 1 glass pineapple and 1 small piece of cake for dinner.

Conclusion

A person may prevent or delay some health problems from CKD by eating recommended foods and avoiding foods high in sodium, potassium, and phosphorus. Proper information about calories, fats, proteins and fluids in allowed food products is important for a person with advanced CKD. Foods rich in proteins such as meat and dairy products break down into waste products that are removed from the blood by healthy kidneys. As CKD progresses, nutritional habits must change. A health care provider, including dietitian, may recommend a patient with reduced kidney function to choose foods very carefully.

References


Nutritional support in a patient with burns

Suzana Atanasovikj*, Bojana Janeku, Dafina Boshkoska, Menka Andreska, Elena Karabeleski, Dragana Mladenovska, Kristina Mladenovska, Lidija Petrushevska-Tozi, Aleksandra Kapedanovska Nestorovska, Tanja Petreska Ivanovska

Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa No. 47, 1000 Skopje, N. Macedonia

Introduction

Metabolism of patients with burns is increased, sometimes even doubled and it takes time to return to normal. Severe catabolism leads to body mass reduction and decrease in immune system function. Increased oxygen consumption throughout the body increases ATP levels and thermogenesis. Reactions that consume ATP are mostly from the hyperbolic response to burns (57%), including ATP for protein synthesis, the production of ATP required for hepatic gluconeogenesis and the glucose and fatty acid cycle (Williams et al., 2009).

Inhibition of the catabolic hormones epinephrine, cortisol and glucagon leads to inhibition of the protein synthesis and lipogenesis. Protein decomposition becomes a necessary and major source of energy, hence skeletal muscle cohesion results with a long-lasting imbalance between protein synthesis and degradation (Clark et al., 2017).

Adequate fast nutritional intervention is very important in preventing complications. Early enteral nutrition reduces circulating catecholamines which preserves intestinal mucosal integrity, motility and circulation, improves muscle mass maintenance and heals wounds (Rodriguez et al., 2011).

This study aimed to illustrate the scale of dietetic activities and to identify nutritional interventions since nutritional support is a critical aspect of the treatment of burn patients.

Case presentation

A 33-years old patient (76 kg, 180 cm) jumped through the window escaping from fire. First aid examination found burns on the extremities, scalp, face, chests and on the back (90% of the body surface). There was a suspicion that the fibulae on the left leg was fractured and that there was an injury of the left wrist joint.

Upon admission in the hospital, oxygen was administered through a mask. Due to suspicion of inhalation burns, he was intubated with 7.5 mm endotracheal tube. Analgesics and intravenous fluids were given and mechanical ventilation was provided. Patient was transferred to Intensive care unit after the fracture surgery was completed.

On the third day of hospitalization, the patient had burns covering 60% of the skin. The body temperature was 38 °C. Laboratory results were: increased bilirubin concentration and transaminases (ALT 900 U/L, AST 700 U/L), increased alkaline phosphatase (680 U/L) and albumin (> 35g/dL), increased prothrombin time, urea 13.2 mmol/L, sodium 116 mmol/L, potassium 6.5 mmol/L. The patient also had another diagnosis, epilepsy treated with oral valproic acid with controlled release.
**Discussion**

The patient had normal value for BMI (23.5). The primary nutritive support for this patient is to meet the increased caloric requirements caused by the hypermetabolic state and to avoid over-eating. Energy needs are changed and fixed formulas usually lead to insufficient food intake for the time of the maximal energy utilization as well as to over-feeding during the later period.

Indirect calorimetry (IC) is a golden standard for measuring the energy consumption, by recording the volume of inhaled and exhaled concentrations of oxygen and carbon dioxide through face mask or by pressurized air cooler. The ratio of produced CO\(_2\) vs. consumed O\(_2\) (VCO\(_2\)/VO\(_2\)) which is influenced by the metabolism of specific nutrients, gives the respiratory quotient (RQ) used to detect overfeeding or underfeeding. Normal metabolism of mixed nutrients yields a RQ of about 0.75-0.90 (Stödter et al., 2018).

Carbohydrates compared to fats are more suitable source of energy at patients with burns, but the glucose oxidation is limited and its need at burned patients is normally increased. Having in mind the possibility that insulin resistance could happen in this kind of injury, patients could benefit from additional insulin therapy. Insulin therapy promotes muscle protein synthesis, wound healing, improves donor healing places, bone mineral density and can reduce spent in the intensive care unit. The optimal maintenance glycemia is 8 mmol/L. In addition, carbohydrates promote wound healing and have a protein like effect. Fats are nutrients needed to prevent deficiency in essential fatty acids but are recommended only in limited quantities. In burns, lipolysis is suppressed and the use of lipids for energy production is also reduced. Increased beta-oxidation of fats provides fuel during the hypermetabolic state, but only 30% of free fatty acids are degraded and re-esterified and later accumulated in the liver. Increased intake of fats may have adverse effects on the immune system and therefore diets with reduced fats intake are generally recommended (fats composition is highly important). The optimal intake is 2 g fats/kg.

Regarding the use of proteins, it is important to mention that proteolysis is significantly increased in severe burns and a patient could lose half a kilogram of skeletal muscle per day. Proteins are a major source of energy but addition of extra calories would not increase protein synthesis, it will lead to overeating. The calorie-protein ratio should be 150:1 in severe burns. Severe burns lead to intense oxidative stress which combined with inflammatory response leads to depletion of endogenous antioxidant deficiency that is highly dependent on micronutrients (vitamin A, E, C, D\(_3\), Fe, Cu, Zn, Se).

**Conclusion**

For the patient with severe burns, enteral nutrition with specific types of nutrients and a customized formula according to the patient's nutritional needs is recommended. Monitoring should include daily weight control and energy intake needs, nitrogen balance, electrolyte balance, fluid-preventing malnutrition needs and muscle mass recovery.

**References**


The role of omega-3 for improvement of mood, behavior and communication skills in children

Elena P. Stanojevska1*, Roberta Mitrevska2

1Nobel Ilac Representative - Skopje, St. 8-th Udarna Brigada No. 39/2, 1000 Skopje, N. Macedonia
2Association for the right of children and youth with special needs “Lastovica”, Blvd. Asnom No. 60-2/18, 1000 Skopje, N. Macedonia

Introduction

The long chain, polyunsaturated fatty acids (LCPUFAs) are well known for more than 100 years. Nowadays, there is a big emphasis on the use of omega-3 and its role for numerous functions in the body. There are two types of PUFAs, omega-6s, which are found primarily in vegetable oils such as sunflower, corn, flaxseed and canola oils, and omega-3s, specifically DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid), two long-chain PUFAs found primarily in fatty fish, and short-chain ALA (alpha-linolenic acid) that comes primarily from plant-based sources like flax. They are crucial for growing infants and critical for development of the brain and central nervous system. DHA is proven essential for pre- and postnatal brain development, whereas EPA seems more influential on behavior and mood. Both, DHA and EPA generate neuroprotective metabolites (Kidd, 2007).

Omega-3 fatty acids are the most studied nutrients in the world. Many people around the world do not consume enough EPA and DHA for brain and heart health support (Murrphy et al., 2015). In 2012, the European Commission authorized an Article 13.1 health claim that min 250 mg per day of EPA and DHA contributes to the maintenance of normal function of the heart, and 250 mg DHA per day contributes for the maintenance of normal brain and eye development. Two-thirds of the human brain is made of fat and DHA as primary structural membrane component makes up to 97% in the brain and 93% in the eyes. The fundamental importance of DHA for brain development is beyond dispute (McCann and Ames, 2005).

Omega-3 vs. omega-6 fatty acids - understanding the difference

Our body does not produce omega 3 and/or omega 6; therefore, we should get them throughout food.

Omega 6 is very much present in today’s modern diet contrary to omega 3. The ideal ratio of ω-3 vs. ω-6 is 1:2. Maximum accepted is 1:4. Nowadays, this ratio goes up to 1:20. Omega 6 fatty acids possess proinflammatory activity, while contrary to this, both DHA and EPA, inhibit the formation of leukotrienes and prostaglandins from arachidonic
acid, and ω-6 fatty acid and reduces the generation of cytokines from inflammatory cells (Lee et al., 1985).

**DHA/EPA proper daily intake may give positive results for improvement of mood, behavior and communication skills in children**

Despite the higher concentration of DHA and EPA needed for more effective therapy for this particular impairment, also the ratio between these two structural similar fatty acids is important as they compete in the gut for absorption. Therefore, the recommended ratio for maximum bioavailability is 1.5:1 for EPA/DHA.

**Materials and methods**

**Materials**

80 children of age from 17 months till 10 years were supplemented with NBL Fish oil (product of NOBEL ILAC -Turkey) for 6 weeks. NBL fish oil is a high-quality purified omega-3 product abundant in EPA and DHA.

**Methods**

Participants were divided in two groups regarding the age. Children of age from 1-4 were given 5 mL per day, while children above 4 years 10 mL. The quantity of 10 mL provides 820 mg of omega 3, out of it 390 mg EPA and 260 mg DHA. The younger group received half concentrations of the daily recommended dosage, respectively for DHA and EPA. A team of specialists were enroled to conduct a careful assessment of the effects of the product on children’s mood, behavior and performances of the communication skills (attention, eye contact, focus, social response).

**Results and discussion**

What triggered our attention was the positive reaction from both parents and health providers in the association during consuming this omega 3 product. The health professionals practice usage of omega 3 as part of children’s treatment for more than 3 years. The omega 3 product had positive influence mainly on the communication skills (short-term possibility for eye contact, managing of the hyperactivity, keeping focus and longer concentration). We are aware that the 6 week supplementation period is very short for a conclusion, but the noted results deserve a chance for longer and further investigation, especially that this is very sensitive, and more often present condition nowadays.

**Conclusion**

There are so many clinical papers that claim the beneficial effects of omega 3, but only few explain the role of concentration of DHA/EPA and their ratio as key factors for positive effects. Research into omega-3 fatty acids as a possible treatment for mood, behavior and improvement of the communication and social skills is new and interesting area of research that should be seriously taken into consideration.

The conclusions are that higher concentrations of EPA and DHA are necessary. Moreover, consumers should pay attention to the origin of the fish oil, the fish source and the ratio of DHA/EPA for higher efficacy of the treatment. The daily intake of omega 6 vs. omega 3 should be as lower as possible and tending to reach ratio of 2:1.

**References**


Consumer perception of risk-benefit of weight loss supplements and building safety

Zoran Zhivikj\textsuperscript{1,}\*, Tanja Petreska Ivanovska\textsuperscript{1}, Marija Karapandjova\textsuperscript{1}, Svetlana Kulevanova\textsuperscript{1}, Marijana Lonchar Velkova\textsuperscript{2}, Lidija Petrushevska-Tozi\textsuperscript{1}

\textsuperscript{1}Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000, Skopje, N. Macedonia
\textsuperscript{2}Consumer Organization of North Macedonia, 50\textsuperscript{th} Division 10A, 1000, Skopje, N. Macedonia

Introduction

Obesity is a medical condition that occurs as a result of the accumulation of fat in the body and it is considered to be a major contributor to a variety of serious diseases. For obesity management, wide spectrum of weight loss dietary supplements is offered globally on the market. Five basic mechanisms of weight loss supplements are involved: controlling appetite, stimulating thermogenesis and lipid metabolism, inhibiting pancreatic lipase activity, preventing adipogenesis and promoting lipolysis (Kazemipoor et al., 2015).

The treatment of obesity using weight loss supplements at an optimum dosage should be safe and effective, but the data are limited in contrast to their popularity and advertisement. Moreover, among the issues that can affect safety, some of the supplements are adulterated with illegal addition of pharmaceutical substances (narcotics, stimulants, antidepressants, anorectics, laxatives and diuretics) or their analogues since unscrupulous producers can falsify these products to provide quick effects and to increase sales (Rocha et al., 2016). Finally, although adulterated with active pharmaceutical ingredients, most often they are mislabeled as being safe and natural (Khazan et al., 2014). In order to gain an insight in the consumer’s experiences related to the use of weight loss dietary supplements in our country, we conduct a survey using an appropriate questionnaire in collaboration with the Consumer Organization of the Republic of North Macedonia.

Materials and methods

A questionnaire composed of 11 questions to objectively collect information about people's knowledge, beliefs, attitudes, and behavior related to the use of weight loss supplements was distributed using the following link: docs.google.com/forms/d/e/1FAIpQLSd3GOwJk17iMtxTCZGPw59ldNx2-M_sUwcvUle0OpiXLYiyPA/viewform?fbclid=IwAR1NJo0xPcfMip19GjL4lRPB7M5umdELn0L_O7rEchoJzUT-2B9_SdXmcWY, provided by Consumer Organization. Total of 242 consumers participated in the survey. Descriptive approach was applied to estimate specific parameters in a population (e.g. where the weight loss supplements were supplied from, frequency of weight loss supplement use, belief in their quality and safety and sufficiency of data provided to people, getting professional advice before consumption, notification of side effects during the use or afterwards).
Results and discussions

The survey confirmed the wide use of weight loss supplements (about 89% of the respondents). Only 16% of them looked for a professional opinion before taking a weight loss supplement. The majority of the people have been governed by the information electronically available or through advertising public media; while only 3% gathered professional help (18% pharmacist’s assistance and 9% contacted nutritionists, health care providers and fitness instructors). About 70% of consumers reported no side health effects during the consumption of weight loss supplements, but they were doubtful regarding their quality. An internet sale was reported to be the most common way of supply, followed by pharmacies and healthy food stores. About 45% of people used weight loss supplements temporary, 35% once in the life and 20% continuously for several months. A great number of people (up to 85%) considered insufficient the available information about efficacy and safety of weight food supplements. Beside the general belief that supplements, especially herbal ones are natural, harmless and effective means of obesity management, an internet sale of such products is not under the strict control and is not always required to pass safety tests before their advertisement and marketing (Khazan et al., 2014; Rocha et al., 2016). With the growing consumption and market globalization of dietary supplements, there is an increased need for accurate and effective screening and structural identification of adulterants or unknown analogues as well as specific regulatory preapproval requirements or safety assessments. Otherwise, consumers may be at great risk of many serious health problems. Even though many supplements do not contain any of the potentially deadly adulterants, many of them contain caffeine and/or green tea extract at such high concentrations that they can also be quite toxic (da Justa Neves and Caldas, 2017).

Despite the potentially serious health risks, very little is known about the prevalence or common adverse effects of dietary supplements adulterated by the illegal addition of pharmaceutical ingredients (Rocha et al., 2016). In our country there is also an increased demand and sale of weight loss dietary supplements, but a lack of data for their quality, efficiency and safety and the real economic profit as well. Hence, relevant authorities should enforce extensive clinical studies for long-term safety and efficacy at the same time strengthening the control tests including screen for contaminants and adulterants before marketing.

Conclusions

People should always seek a professional advice for proper use of weight loss supplements. Health care professionals are obligated to provide all necessary information and make people aware of related risks. Both, the research community and regulatory agencies are entailed to establish appropriate methods for detection of possible adulterations in weight loss supplements in order to protect the public health.

References


Whose recommendations supplement users believe the most?

Suzana Miljković

Faculty of Pharmacy - Novi Sad, Trg mladenaca 5, 21000 Novi Sad, Serbia

Introduction

Food supplements are very attractive products for consumers of all age groups, with different life styles, health status, and various needs, and their use increasing among many populations in the world (Perlitz et al., 2019). According to the Directive 2002/46/EC: “food supplements are foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose forms - capsules, pastilles, tablets, pills etc. designed to be taken in measured small unit quantities”. These products are available in pharmacies and various shops, and consumers are freely to decide which product to choose and how to use it.

Information on their effects, properties, possible side effects and interactions with the drugs, food or other supplements, as well as suggestions on their selection and application are available from different sources: healthcare professionals (medical doctors, pharmacists), relatives, friends, media, social networks, etc. As this category of food is growing every year, and many new ingredients are introduced, a lot of them having a natural origin, consumers need to be properly informed. Due to application in pharmaceutical dosage forms, many users believes these products act as a drugs, it is important to encourage only the realistic expectations.

In order to achieve the desired outcomes of food supplements use, consumers should follow the healthcare professionals’ advice. Pharmacists, with their professional expertise on the selling point, are well educated to give the proper recommendation for the selection, application and expected outcomes of the food supplements according to the needs of any user (Goundrey-Smith, 2018).

In this study, we analyzed the choice of sources of information of participants in 10 separate studies dealing the various food supplements use in Serbia, as well as the influence of pharmacist’s advice on the consumers.

Materials and methods

This is cross-sectional study of 10 separate investigations on food supplements use, realized in several cities in Serbia. We analyzed only the results on one question in purposefully made questionnaires used in these studies: what are the most influential sources of information for the participants. Offered options were: doctor, pharmacist, relatives and friends, media and social media, others. We pay special attention on influence of pharmacist’s advice on the consumers.

Results were presented in %, as an average value of total number of participants.
**Results and discussion**

There were total of 1459 participants, mostly women (65.7%), with secondary level of education (52.9%) and an average of 44.4 years. According to these results, women were more interested in their and the health of their family, they take more care about the use of supplements and more often than man buy these products. The studies were realized in Novi Sad, Belgrade, Krusevac, Požarevac and other cities in Serbia in the period 2017-2019. The food supplement categories, investigated in this 10 studies were: for immunity, dislipidemia, pregnancy and lactation, eyes, weight loss, products with zinc, magnesia, phytonutrients, antioxidants and probiotics.

Most of the respondents have chosen the recommendations of doctors (38.5%) and pharmacists (28.2%) as relevant for selection of this kind of products. This means ⅔ of all respondents have chosen the advice of health care professionals, and we could say the recommendations they got were evidence-based and according to the exact needs of every consumer. This is the best choice, as there are many other sources of information where some important data could be omitted, or generated on the basis on irrelevant origin. Advice of gynecologists (67%) and ophthalmologists (80%) were the most important for users of food supplements for pregnant and lactating women, and eyes, respectively. This is quite understandable, as these consumers need the support for their regular treatment/therapy and asked for the physicians’ opinion.

Relatives and friends were the chosen sources of information for 15.7% participants, media (printed, electronic media, social networks, etc.) were chosen by the 11.4% and others by 6.3% participants. As relatives and friends recommended products on the basis of their previous experience with effects of the product on their own problems, this kind of suggestions shouldn’t be crucial for the decision. Anyway, their opinion was important for supplements with zinc (28.3%) and phytonutrients (aronia) (28.3%) users. Influence of media (TV, newspapers, internet) was important for consumers of antioxidants (26%), and as a second choice, beside the doctor’s advice, for pregnant and lactating woman (20%). As a younger group of participants (23-40 years old) it is expected they were looking for the information in media, but it was very important to discuss these data with their physicians, too.

Pharmacists’ suggestions were the most important for 28.2% of consumers, mostly the users of food supplements for immunity (47.6%), dislipidemia (40%), and with magnesia (37%). Supplements that support immune functions are among the best seller products, and their consumers often take them for “just in case” reason. Similarly, magnesia supplements are used for multitude of reasons, and the offer of this group of products is huge, no wonder users need a professional advice. Anyway, we believe the influence of pharmacists, and their recommendations on food supplements’ consumers should be more pronounced. Through the communication and proper counseling the users, pharmacists should gain more interest and trust of the consumers.

**Conclusion**

Most participants of the studies used in this analysis, chosen the health care professionals as an adviser in food supplements selection and use. Although not quite relevant, an opinion of relatives, friends and media were also important for some respondents. Beside the doctors, pharmacists were the second most trustworthy source of information, but they should try to gain more trust by the consumers.

**References**


Synthesis and antioxidant activity of newly synthesized chalcones

Milica Tasić¹*, Nemanja Turković², Branka Ivković¹, Jelena Kotur-Stevuljević³, Zorica Vujić¹

¹Department of Pharmaceutical Chemistry Faculty of Pharmacy, University of Belgrade, 11000 Belgrade, Serbia
²Agency for Medicines and Medical Devices of Montenegro, 81000 Podgorica, Montenegro
³Department of Medical Biochemistry Faculty of Pharmacy, University of Belgrade, 11000 Belgrade, Serbia

Introduction

Chalcones (1,3-diaryl-2-propen-1-ones) are natural products abundant in fruits, vegetables, spices and teas. More recently, chalcones are obtained synthetically by the reaction of aldol condensation (Claisen-Schmidt condensation). Chalcones have a wide range of biological activities: antiinflammatory, antioxidant (Halliwell and Gutteridge, 2006), antibacterial, antifungal, antiproliferative (Dimmock et al., 1998) and others, as well as low selectivity that limits their clinical application.

Materials and methods

Synthesis of chalcones

Compounds used in synthesis were mono- and disubstituted halogenated benzaldehyde which gave the corresponding chalcones in the reaction with the mono- or disubstituted acetophenone. Chalcones were synthesized in the reaction of base catalysed aldol condensation.

Structural analysis of synthesized substances

The structures of compounds were determined by infrared spectroscopy (IR), nuclear magnetic resonance (NMR) and mass spectroscopy (MS). Melting temperatures of newly-synthesized chalcone derivatives were determined by the instant melting method.

Antioxidant activity assay

The antioxidant activity was tested in serum of healthy adults by determining the following parameters: TAS (total antioxidant status), TOS (total oxidative status), PAB (prooxidative antioxidant balance), SHG (sulphydryl group content) (Erel, 2005). Based on these values, Z-scores of protoxidative (TOS+PAB) and antioxidative (TAS+SHG) parameters were calculated, and from their difference, an oxidative score was obtained as a measure of antioxidant activity. An oxidative score was also determined in serum samples in which except chalcones, the tert-butyl hydroperoxide (TBH), strong synthetic prooxidant was added and in pure serum as well.

Results and discussion

Chalcones were synthesized by Claisen-Schmidt condensation and after their physicochemical characterization 18 derivatives of chalcones that showed a high degree of purity after purification...
were selected and were tested for antioxidant activity. When the oxy scores were calculated for all compounds, the obtained values were statistically processed in the SPSS program.

The oxidative score of certain chalcones was in range of 9.56 to 283.67 (133.93 ± 83.06), while for the group of chalcones containing two phenol groups in its structure, oxidative score was in range of -44.07 to -151.62 (-89.13 ± 40.87).

As the oxy score is an indicator of strength, i.e. capacity of an antioxidant activity of a compound, compound having a negative value of this score will exhibit significant antioxidant activity. Such values were observed in the second group of compounds which differ from the first group in terms of structure in the presence of another phenolic group in the ortho position. This is confirmation of previous studies where hydroxy chalcones have been shown to have a potent inhibitory effect on the formation of superoxide anions in rat's neutrophils (Ni et al., 2004).

The steric influence of the substituents was also shown (Kim et al., 2008) and the substituents in the ortho and para positions had a negative effect on the antioxidant value as opposed to the substituents in the meta position.

As demonstrated in the study, electron-attracting groups reduce the efficiency of hydrogen release from the amino group of pyrazolines and therefore reduce the antioxidant activity (Akshay et al., 2013).

**Conclusion**

Analyzing of all obtained data, it is concluded that chalcones have antioxidant activity whose potential depends on the substituents as well as their position in the molecule (Batovska, 2010).

ANOVA test showed a statistically significant difference between the oxidative scores of the group of chalcones with one and two phenol groups (p<0.05), what indicates that there is a significant influence of the presence of several phenol groups in the compound on the antioxidative activity of chalcones.

Besides this, substituents such as halogens and other electron distractors should be avoided. Meta position of the substituent has the highest contribution to the antioxidant activity.

**References**


Synthesis and pharmacological trials of new phosphorylated oxazole derivatives antihypertensive properties

Iryna V. Nizhenkovska1, Kateryna V. Matskevych1*, Oleksandr V. Golovchenko2, Oksana I. Golovchenko1

1Bogomolets National Medical University, T. Shevchenko boulevard 13, 01601, Kyiv, Ukraine
2Bioorganic chemistry and petrochemistry institute, Murmanska str. 1, 02094 Kyiv, Ukraine

Introduction

1,3-oxazole derivatives are known to exhibit a wide range of biological effects. They are a part of natural bioactive molecules and synthetic drugs (Harris et al., 2005; Niraimathi et al., 2011). 5-amino-1,3-oxazoles are often regarded as masked peptides, so such substances are perspective pharmacophore groups for modification of the peptide chain. In addition, dehydroamino acids are of great interest among biochemists and pharmacologists. Therefore, the synthesis of novel compounds of peptide nature, which contain fragments of dehydroamino acids and 5-amino-1,3-oxazole ring, is promising for further pharmacological studies as new bioregulators of various actions.

Phosphorylated oxazole (POD) derivatives are one of the perspective groups of this type of compound with vasodilatation properties. Only sporadic studies of the vasoactive action of this group of compounds in vitro have been identified in the literature, which have not yet been investigated in vivo (Iakovenko et al., 2013).

The above was the basis for pharmacological study of the antihypertensive action of these substances with the prospect of their recommendations for further study and use as medicines for the treatment of hypertension.

Materials and methods

POD synthesis

POD synthesis was performed using 5-amino-2-phthalimidoalkyl-1,3-oxazol-4-ylphosphonic acid diethyl esters.

Screening for vasodilator properties of POD in vitro

Screening of vasodilator properties of a number of PODs and determination of a leader compound was performed on isolated segments of the aorta of rats in vitro (Mikkelsen and Pedersen, 2001). Vascular reactions were recorded using Iris Waveware software for a USB oscilloscope («Iris», CIIIA).

Investigation of the antihypertensive effect of a leader compound of POD (OVP-1) in vivo

The influence of the POD leader compound on blood pressure and hemodynamic parameters was performed on an adrenaline model of acute hypertension with single intravenous (i/v) administration to rabbits of both sexes. The criterion
for effectiveness was the decrease in mean blood pressure (MBP) after the introduction of OVP-1 and modeling of acute hypertension by not less than 25 mm Hg compared to MBP in the blank group (Stephanov, 2001).

**Results and discussion**

The possibility of using derivatives of diethyl esters of 5-amino-2-phthalimidoalkyl-1,3-oxazol-4-ylphosphonic acids (I) for the synthesis of phosphorylated oxazole derivatives is shown. When exposed to the compounds (I) of hydraying hydrate, diethyl esters of 2-aminoalkyl-5-alkylamino-1,3-oxazol-4-ylphosphonic acids (II) are formed. By the interaction of oxazoles (II) with unsaturated azlactones POD, containing fragments of dehydroamino acids, are synthesized. The developed synthesis method is convenient and preparative, since the transformations take place under mild conditions, which avoids the formation of unwanted side-products, and the POD is obtained in high yields without the use of chromatographic columns.

According to the results of screening studies in *vitro*, it was found that in isolated segments of the descending part of the thoracic aorta of rats, a compound-leader in a range of phosphorylated oxazole derivatives OVP-1 - OVP-10 was OVP-1, which in concentration $1\cdot10^{-5}$ M most clearly reduced the force amplitude of the adrenaline-initiated $(5\cdot10^{-6}$ M) by 30.9% (P<0.05) relative to the baseline.

As a result of *in vivo* studies, it was found that the criterion for reducing blood pressure in the adrenaline model of acute hypertension was achieved with the introduction of OVP-1 at a dose of 25 mg/kg, which corresponds with the ED$_{50}$ of the test compound under these conditions of administration 24.19±2.98 mg/kg and at a dose of 50 mg/kg (2ED$_{50}$).

It was found that the antihypertensive effect of 25 mg/kg OVP-1 substance is due to its effect on the reduction of total peripheral vascular resistance by 40.9 % (P < 0.05) relative to baseline and left ventricular working index by 45 % (P<0.05) relative to the blank group after adrenaline administration.

**Conclusion**

The obtained results are the basis for the purposeful synthesis of potential antihypertensive agents based on phosphorylated oxazole derivatives and for further in-depth preclinical and clinical studies to develop a new original antihypertensive drug to prevent the development and treatment of arterial hypertension.

**Conflict of interests**: None

**References**


Short communication

Synthesis, molecular modeling and evaluation of anticancer activities of some 1-substituted-4-phenyl piperazine derivatives

Mehmet Abdullah Alagöz¹, Zeynep Özdemir¹, Ceylan Hepokur², H. Eren Bostancı², Tijen Önkol³*

¹Inönü University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 44210 Malatya, Turkey
²Cumhuriyet University, Faculty of Pharmacy, Department of Biochemistry, 058140 Sivas, Turkey
³Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 06330 Ankara, Turkey

Introduction

Cancer is a disease caused by the disruption of the cell cycle, which is controlled by the cell's genetic material. Chemotherapy, radiotherapy and surgical methods are used as standard in cancer treatment. In chemotherapy, tumor cells are targeted to destroy or stop their growth. Due to the fact that the existing anticancer drugs do not have sufficient effect and they have various side effects, new anticancer drugs are needed to be developed (Herrero and Medarde, 2015; Zhang et al., 2014). In this study, three new 1-substituted-4-phenylpiperazine derivatives, which are expected to have anticancer activity, were designed, synthesized and anticancer activities were investigated.

Materials and methods

Molecular modeling studies

Maestro 11.8 (Schrödinger, LLC, NY) program was used in molecular modeling studies. Three-dimensional structures of compounds were constituted with Maestro11.8 software with the aid of MacroModel software and the OPLS_2005 force field parameters, and were optimized by conjugated gradient method. The structure of HSP90 protein (PDB ID: 1YET) retrieved from RCSB (www.rcsb.org) for C6 and MCF-7 (Dogan et al., 2017).

Chemistry

Synthesis of compounds begins with 2-acetylnaphthalene. 1-(Naphthalen-2-yl)-2-bromoethanone is obtained as a result of the bromination reaction (Immediata and Day, 1940). Compounds 1a, 1b and 1c were synthesized by reaction of this compound with 1-phenylpiperazine, 1-(3-(trifluoromethyl) phenyl) piperazine and 1-(2-methoxyphenyl) piperazine, respectively (Ozdemir et al., 2015). The structures of the obtained compounds were illuminated by spectral methods (¹H-NMR, ¹³C-NMR, IR).

Activity studies

MCF-7 (breast cancer cell line), C6 (rat brain glioma adenocarcinoma cell line) and WI-38 (healthy human fibroblast cell line) were used in activity studies. In these studies, 5-fluorouracil (5-FU), an anticancer drug, was used as the reference compound. The IC₅₀ value of 3 synthesized
compounds and 5-FU was determined by XTT (Chiang et al., 2006).

**Results and discussion**

The interactions of the compounds with residues in the active region of the HSP90 protein were examined and docking scores were calculated. The compound with the best docking score is 1c. In the study, the 1c was observed to be more active in the C6 cell line than 5-FU; 1b in MCF-7 cell line was found to have an activity close to 5-FU.

It was determined that the docking scores obtained from molecular modeling studies are in harmony with the activity studies.

**Conclusion**

In this study, it was determined that the molecules synthesized according to the results obtained from molecular modeling studies have significant anticancer activity. It also has low cytotoxic effects against healthy cells. Due to these high effects and properties, these molecules become potential anticancer drug candidates. Advanced *in vitro* and *in vivo* preclinical and clinical studies are needed for compounds to become candidates for anticancer drugs.

**References**


Evaluation of acute intraperitoneal toxicity of new germanium coordination compounds

Ihor Kryvoi*, Iryna Nizhenkovska, Violetta Narokha, Olena Kuznetsova

Bogomolets National Medical University, Department of Pharmaceutical, Biological and Toxicological Chemistry, Pushkinska str. 22, 01004 Kyiv, Ukraine

Introduction

Complex compounds of metals with bioorganic ligands is one of the promising areas for the search for new active substances with a high safety profile, which can subsequently form the basis of new medicines. Such substances include complex compounds of germanium with organic acids, which already have shown antioxidative, membrane-, neuro-, hepato- and cardioprotective effect in a range of studies (Kresyun et al., 2004; Narokha et al., 2016).

To study the safety and evaluate the toxicity of new compounds, an important step is the determination of acute toxicity in rodents, which makes it possible to estimate the feasibility of further studies of the compounds, adequately select doses for possible subsequent studies and draw conclusions about possible damage to organs and systems.

Aim

The goal of this study was to determine the acute intraperitoneal toxicity of three new germanium coordination compounds (OE-3, OE-6, OE-7) by estimation of its median lethal doses (LD_{50}) on mice, identify its toxic effects and classify due to toxicity class.

Materials and methods

Germanium coordination compounds with oxyethyliden diphosphonic acid and nicotinamide (OE-3, OE-6, OE-7) were synthesized in laboratory of Department of General Chemistry and Polymers of Odessa I.I.Mechnikov National University under the supervision of professor Seifullina I.I.

The study was conducted in adult (8-12 weeks) CD-1 mice, weighing 18-22g. The animals were maintained (including euthanasia) pursuant to the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (2016/63/EU). The animals were fed balanced diet and had free access to water in the animal house (vivarium) of the Bogomolets National Medical University (Kyiv, Ukraine). Animals were kept in their cages for one week before the beginning of the experiment for acclimatization to the laboratory conditions. Individual weights of mice were determined before germanium compound administration and weekly thereafter. Each dose of each substance was administered intraperitoneal as a single dose to groups of seven mice. A dose range was selected as five dose levels with an upper limit of 1000,0 mg/kg of body weight for each substance (62,5; 125,0; 250,0; 500,0 and 1000,0 mg/kg) which corresponds to the maximum dose for the IV toxicity class which is limiting for an acute toxicity study (Stefanov, 2001). After injection of germanium...
complexes, mice were observed with special attention during the first 4h and daily thereafter, for a total of 14 days generally. Signs of toxicity and mortality were recorded.

The LD50 values based on mortality data, were calculated according to the probit analysis method and method of Litchfield and Wilcoxon at the 95% confidence level.

**Results and discussion**

Obtained results of mortality rate and toxicity after substances administration shows that death of mice injected with OE-7 occurred starting from the dose of 125 mg/kg while death of mice injected with OE-3 or OE-6 occurred starting from the dose 250 mg/kg. LD50 values of OE-3, OE-6, OE-7 were ranged between 300 and 400 mg/kg of body weight, which corresponded IV class of toxicity – slightly hazardous substances and also corresponded the literary data on the toxicity of germanium compounds (Godovan, 2008; Narokha, 2018). According to the degree of toxicity substances are located as follows: OE-7>OE-6>OE-3.

With the substances administration, rapid developments of toxic effects were observed. In mice, there was a reduced activity, lethargy and, in case of mortality, dyspnea, tachycardia and tonic seizures: a constant muscle contraction accompanied by persistent extension of the hind limbs. Moreover, the symptoms of OE-7 were more pronounced, which is probably due to the greater toxicity of this substance.

Death predominantly occurred within an hour after the introduction of substances and if the rodent survived, after 24 hours it felt cheerful. Such effects indicate the toxic effects of high concentrations of germanium complexes on the central nervous system and neuromuscular innervation.

There were no significant changes in weight in animals recorded.

**Conclusions**

Three new germanium coordination compounds with bioligands (OE-3, OE-6 and OE-7) referring to IV toxicity class – slightly hazardous substances, LD50 of these compounds lies in the range 300-400 mg/kg of body weight, which confirms their safety and the feasibility of further animal studies using these compounds.

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In silico analysis of monoamine oxidase B inhibitory activity of 8-substituted xanthine derivatives

Iva Valkova¹*, Javor Mitkov², Maya Georgieva², Alexander Zlatkov²

¹Department of Chemistry, Faculty of Pharmacy, Medical University - Sofia, 2 Dunav st., 1000 Sofia, Bulgaria
²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University - Sofia, 2 Dunav st., 1000 Sofia, Bulgaria

Introduction

The monoamine oxidase B (MAOB) is a mitochondrial enzyme that catalyzes degradation of neurotransmitter amines. MAOB inhibitors increase the dopamine levels and are frequently used as a supplement treatment of levodopa therapy in Parkinson’s disease. They also exert a neuroprotective effect by reducing the excess formation of hydrogen peroxide and aldehydes that are neurotoxic (Youdim and Bakhle, 2006). In last decades there is a steady interest in the design of inhibitors by modification of naturally occurring compounds, including caffeine (Dhiman et al., 2018). Although it is a weak MAOB inhibitor, 8-substituted xanthines have shown improved inhibitory profiles (Booysen et al., 2011). In the current study we developed quantitative structure-activity relationships (QSAR), based on literature data for MAOB inhibitory properties of 95 derivatives (Mostert et al., 2012; Okaecwe et al., 2012; Strydom et al., 2010, 2011).

The aim was to reveal the structural characteristics, most relevant for MAOB potency of compounds and to guide a future synthesis.

Materials and methods

Biological activities of compounds

MAOB inhibitory activities of compounds were collected from the literature. They were measured as 50% inhibition concentration (IC₅₀) in the same laboratory, following the same experimental protocol, which is necessary condition for reliable analysis. For the purposes of QSAR, activity was expressed in pIC₅₀ units (pIC₅₀ = -log IC₅₀) thus stronger inhibitors acquired higher pIC₅₀ values and vice versa.

Descriptors of the molecular structure

Four domains for structural modifications were distinguished in the molecules of investigated compounds. The first is located in the xanthine -N1 and N3 atoms. The rest three concern the side chain – atom connected at C8 position, terminal substituent, opposed to xanthine and the bridge in between. Altogether 36 different fragments were identified and they served as molecular descriptors. Structures were binary coded by indicator variables – 1 for presence and 0 for absence of the respective fragment.

* ivalkova@abv.bg
Model development and validation

The descriptors most relevant to MAOB inhibitory activity of compounds were selected by genetic algorithm, as implemented in the package MDL QSAR v.2.0. Multiple linear regression models were derived and estimated on the basis of determination coefficient ($r^2$), standard error of estimate (SEE) and Fisher coefficient (F). The ability of models to predict activity of non-synthesized compounds was checked by cross-validation and y-scrambling procedures.

Results and discussion

The best linear regression model for structure-activity relationships outlined modifications in the side chain as more significant for MAOB inhibition compared to these in the xanthine ring. It showed positive correlation between the presence of oxygen or sulfur atoms connected to xanthine C8-atom and MAOB inhibitory activity. Two descriptors, accounting for properties of the linker, participate in the equation. These are bridge, consisted of four methylene groups and inclusion of additional oxygen atom. Both are favorable for the antagonistic activity, because possesses positive correlation coefficients. Six descriptors describe the contribution of the terminal fragments. Meta and para, chloro- or bromo-substituted phenyls cause the greatest improvement in MAOB inhibition, while saturated aliphatic (isopropyl) cyclic (cyclopentyl) derivatives are among the weakest inhibitors.

The correlation plot between experimental and predicted pIC$_{50}$ values for investigated compounds revealed four outliers, for which a deviation of more than one logarithmic unit of the predicted pIC$_{50}$ is detected. These are compounds with short or missing linker, containing also cyclohexyl or unsubstituted phenyl rings and the model poorly predicts their activity.

Results of the cross-validation and randomization procedures showed the model is able to reliably predict MAOB inhibitory activity of non-synthesized compounds.

Conclusion

The present study revealed the structural features of 8-substituted xanthines favorable for their MAOB inhibitory activity, namely sulfur and oxygen atoms, directly connected to the C8 atom from the xanthine ring, meta and para chloro- and bromophenyl terminal substituents, elongation of the linker as well as introduction of second oxygen atom in the side chain. Ten new xanthine derivatives were designed, synthesized and submitted to an in vitro testing for MAOB inhibition.

Acknowledgements

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References


Innovative medicines & managed entry agreements; a happy marriage?

Tanja Fens\textsuperscript{1,2}, Maarten J. Postma\textsuperscript{1,2,3,*}

\textsuperscript{1}Department of Health Sciences, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
\textsuperscript{2}Institute of Science in Healthy Aging & healthcare (SHARE), University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
\textsuperscript{3}Department of Economics, Econometrics & Finance, University of Groningen, Faculty of Economics & Business, Groningen, The Netherlands

Access to innovative medicines

One current bottleneck concerns the access and reimbursement of innovative medicines, related to uncertainty about effectiveness and safety, but primarily conceived high prices. Moreover, there is an unmet medical and societal need for access of innovative medicines in the developing countries (Inotai and Kaló, 2019). Contemporaneous, all parties involved in the medicine pathway are facing their own challenges. Common ground to overcome the differences and fulfill the needs of all participants in one integrated healthcare system may be the design of Managed Entry Agreements (MEAs).

Managed Entry Agreements

A MEA is an innovative tool engaging health authorities and pharma-industry to reach agreements towards facilitating the access to innovative medicines and structure price negotiations as well. Using various instruments, MEAs often ensure minimalization of uncertainty from economic evaluations and budget impact analyses accounting for the real-world evidence (RWE) (Ferrario et al., 2017; Kanavos et al., 2017). Notably, the recent decade has shown a strong trend of including RWE in the assessments, next to obviously still the data from trials. Furthermore, there is fast change and wide range of reimbursement policies across Europe. In conjunction with conventional HTA, national authorities are using various contracting schemes, such as price cuts, reference price policies, budget caps, patient co-payments, payment by result, coverage with evidence development, price-volume agreements, discount agreements, agreements for free doses, payback agreements and conditional reimbursement (Wenzl and Chapman, 2019). Notably, all these contracts exemplify MEAs.

Types of MEA

MEAs can be financial- or health outcome-based agreements. The latter might be based on performance or evidence development. Financial agreements are increasing the financial certainty through price based on volume or market share, total spend, or a combination of both at population level, free treatments and fixed price or cost-sharing at the patient level. Health outcome-based agreements are supposed to cope with the uncertainty in the outcomes with risk-sharing, can be related to

* m.j.postma@rug.nl

\textsuperscript{*} m.j.postma@rug.nl
adherence, cover performance payment at population level or additional patient services and ensure shared accountability or evidence-based outcomes at the patient level. Finally, a third type of MEAs exist, sharing characteristics of both types previously mentioned. (Wenzl and Chapman, 2019)

**MEA in practice**

Globally seen up to 2018, Europe is a leader with more than 60% of MEAs submissions. Within Europe, Italy dominates with 88 innovative contracting submissions, followed by the UK (70), and Sweden (68). (Deloitte, 2019) When MEA-type is concerned, preferred use of financial-based agreements is noted in Belgium, Lithuania, England, Portugal, Malta, Cyprus, while the performance-based ones were more common in the Netherlands, Sweden and Czech Republic. (Kanavos et al., 2017)

The latter are concerned with coverage-under-evidence-development. Notably, monitoring processes are not transparent and primarily focused on effectiveness, which demonstrates need for future attention on safety as well. Obviously, the field of MEAs is an extremely dynamic field, for example, the Netherlands recently moved away from performance to price negotiations. (Wenzl and Chapman, 2019) The utilization of MEAs in Central and Eastern Europe is mostly dominated by discounts (73%), paybacks (14%), price-volume agreements (5%), free doses, and bundle agreements (< 5%). (Ferrario et al., 2017) These agreements are mainly focusing on oncology and diabetes medicines. Even though the implementation and utilization trend is increasing, there is a lack of transparency noted in these countries. (Ferrario et al., 2017; Inotai and Kaló, 2019).

**Future implications & conclusion**

Access to innovative medicines, personalized treatments and improved medicine performance, affordability within the budget and effectiveness uncertainty, as well as impetus for further research into innovative medicines, all reflect advantages justifying the use of MEAs. Yet, further work on MEAs is required. Notably, further development in the area of transparency in pricing and costing are needed as well as solutions for medicine access after agreement expiration, simplifying and setting a standard agreement framework, improved patient and data monitoring mechanisms with attention on safety outcomes, sharing information from performance-based MEAs and developing methodologies towards measuring societal value for continues use of gathered data in HTA. (Kanavos et al., 2017; Wenzl and Chapman, 2019)

In conclusion, increased and systematic use of HTA in correlation with MEA facilitates the access to innovative medicines and represents a good informative tool for the decision makers.

**References**


National medicines policy in Poland - pharmacoeconomic and IT solutions

Marcin Czech

Department of Pharmacoeconomics, Institute of Mother and Child, Kasprzaka str. 17A, 01-211 Warszaw, Poland

Introduction

Pursuant to Art. 68 of the Polish Constitution, everyone has the right to have their health protected and the state is obliged to ensure equal access to healthcare services financed from public funds to all citizens. For the first time in many years, the Polish government has decided to treat health care as its priority. It has been recently decided by the government to increase spending on health care to 6% of GDP in 2024. The planned nearly two-fold increase in healthcare expenditure is a historic moment. The goal now is to make the most efficient use of the money to secure tangible benefits for patients and help satisfy the health needs of citizens in the best possible way.

The role of decision makers is to ensure an efficient healthcare system which will facilitate the prevention of diseases and provide the best care and pharmacological treatment to those who are already ill.

Materials and methods

“National Medicines Policy in Poland 2018-22” was drafted on the basis of the 2016 guidelines of the World Health Organizations on how to develop and implement national drug policies (WHO, 2016). It lays down mid-term and long-term goals set for pharmaceutical market participants and decision makers and identifies the main tools required to achieve them. The document was developed in the course of a structured process of consultations with all stakeholders. The following parties took part in the creation of this document: representatives of the public sector, including the Ministry of Health (coordinator of the process); Ministry of Entrepreneurship and Technology; Ministry of Finance; Ministry of Family, Labour and Social Policy; National Health Fund; Agency for Health Technology Assessment and Tariff System; Chief Pharmaceutical Inspector; Chief Sanitary Inspector; National Medicines Institute; National Health Institute; and the Office for Registration of Medicinal Products, Medical Devices and Biocidal Products; MPs and senators who are members of parliamentary Health Committees; representatives of patients’ organizations; associations of pharmaceutical industry manufacturers and employers, and self-governing authorities of the medical professions. The objectives of the National Medicines Policy are consistent with the Commission on Essential Medicines Policies published in 2017 (Wirtz et al., 2017). In each of the covered areas, the document provides a description of the status quo, a diagnosis and description of the characteristics of the key challenges. It also sets the objectives and describes the tools required to achieve them as well as the methods of performance measurement. Additionally, it addresses the need to strengthen the position of the pharmaceutical industry based in Poland and the special role of the
e-health system in the future. In view of the growing importance of prophylaxis and the need to prevent diseases, it also includes primary prevention section. The document was reviewed by WHO.

**Results and discussion**

The National Medicines Policy is a strategic document, which sets out the priorities of the Government of the Republic of Poland regarding the management of medicines between 2018 and 2022. It also responds to the expectations of various stakeholders to set forth the framework and directions for medicines management.

Patients should have access to medicines with proven efficacy, quality and safety for the prevention and treatment of diseases, both in inpatient and outpatient settings. At the same time, in order to ensure sound management, medicines financed from public funds should meet the requirements of cost-effectiveness and their financing must be within the current limits of the payer’s budget according to pharmacoeconomic principles. The objective is to increase the accessibility of medicines in order to satisfy the health needs of patients to the greatest extent possible. This goal can be achieved via a methodical increase in the number of cost-effective and affordable medicines and a reduction in the patients’ co-payments as well as by speeding up the time to market of new medicines.

Reimbursement decisions under which medicines are co-funded from public sources are made on the basis of epidemiological and demographic data and public health needs, within a transparent decision-making process. From the social perspective, population groups which require special attention include children, pregnant women, persons with disabilities and seniors. The Medicines Policy is informed by scientific evidence and reliable data, in particular concerning such aspects as mortality, morbidity and prevalence, risks, medicine efficacy, safety, quality and cost-effectiveness, demographics, productivity, incapacity for work, disability, costs and micro- and macro-economic indicators.

Poland supports measures aimed at enhancing the competitiveness and innovativeness of the pharmaceutical industry by encouraging research and development of new medicines, improving the conditions for conducting clinical trials, increasing the production capacity in Poland, fostering export sales and facilitating international expansion.

The National Medicines Policy should be implemented through adopted legislation and pursued by the competent public authorities. The goals will be regularly reviewed, both in terms of the impact on the general population’s health and on the national economy.

**Conclusion**

The guidelines of the World Health Organizations create an excellent framework for the development of strategic state documents covering medicines management. The Medicines Policy should always be an integral element of country’s health policy.

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Changes in the R&D process and consequences on drug pricing

Rubin Zareski

Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, North Macedonia

Introduction

A new paradigm of the so-called “Circular economy and resource efficiency” economy is evolving. Optimisation, sustainability, long term efficiency, customization are becoming widely spread pillars of a new development Models. Pharmaceutical business is not spared from these systematic changes. Moreover, the pharmaceutical industry, regulator, funds, payers and patients are under growing pressure to adopt very fast to these emerging needs. Old pricing methodologies rely to a significant extent to costs and consequently on price reduction affecting all parts of supply and delivery chain. In the eyes of patients and other stakeholders, the pharmaceutical industry exists to discover new medicines that go on to become standard treatments. The faltering economics of R&D productivity are jeopardizing that mission: R&D expenditure is not delivering. Although number of facts are saying that now is the right time (maybe also last chance) to institutionalize the process of the “holly trinity”, R&D, HTA, reference pricing methodology, major dilemma is whether the approach should be driven by market forces or by regulations, or by mix.

Redefined or revolutionized approach

Healthcare consumes 12% of global GDP, and is a $6.2 trillion industry. Spend is growing at average of 3.5% per annum, which will double expenditure in less than 20 years. By 2020, chronic diseases will account for almost three-quarters of all deaths worldwide. Moreover, it is estimated that by 2030 more than 40% of the world population will suffer from one or more chronic disorders. By 2080, estimated 50% of world GDP will be spend on health costs (OECD analysis). The health care system is under growing pressure as a result. Unless we intervene at short notice in a number of different domains, financial and staffing shortfalls will be inevitable. To ensure that health care system is future proof, we need a new perspective that corresponds more closely to people personal experience.

This route has three game changers. First is to acknowledge the health as a basic condition for participating, playing active role in society a different and broader view than the prevailing biomedical model, which focuses on sickness. Introduction of the concept of “positive health”, in which actions are guided by the desires, values and preferences of the individual needs, is to become a focal point. Second, actions of all involved participants must be focused on the notion of QUALY that should replace the limited approach of what someone with an illness is still capable of. Third, to implement the concept of positive health, we must be more knowledgeable and aware of variations between individuals: variations in norms, values and goals, but also in lifestyle, behaviour, environment, genetic disposition, and above all in the body’s response to healthy and pathogenic stimuli.

There are two possible alternatives in addressing this situation. First are the so called “Pragmatic
trials” which provide fast and efficient information for decision making by providing evidence for the adoption of treatments into practice (Ford & Norrie, 2016). Carefully constructed and executed pragmatic trials provides the bridge between research performed for the development of treatments to the evaluation of the effectiveness of treatments in practice. Second solution is to outsource the R&D to the more efficient, focused, equipped companies and to introduce pay-for-performance Model.

In this new environment, pharmaceutical companies will have to make major shift in the policy implementation by trading price for access to markets. On the other side Governments on the long run in finding a right balance will need to introduce pharmacoeconomic studies. To make this new approach possible beside the large-scale comparative valuable studies that form the basis of medicine today new research methods and outcome measures will focus on valid and sensitive measures for many disorders that allow us to track and predict outcomes to supplement existing methodologies. What is vital for future R&D is a broad, interdisciplinary approach in which the public/patients, pharmaceutical organisations and co-funding bodies also play an important role mainly by investing in sickness prevention. We should also be studying and applying the concept of personalised prevention. It is also important to research how prevention can be integrated permanently into the social fabric.

New R&D model

Considering the fact that about 15% to 20% of clinical spend is wasted on efforts that are not valued by key stakeholders, pharmacoeconomists need to focus on:

1. continuing analysis, including assessment of the critical drivers of behavior for each stakeholder (prescriber, pay or/and patient). One very important and specific prerequisite is to have an advanced data infrastructure consisting of existing and new studies and documentation.

2. detailed comparison of competitor labels and clinical data- to further inform which efficacy and safety endpoints matter most, and maps each of the brands against the stakeholder perception

3. understanding of how competitors are perceived by each stakeholder against the most critical factors, which can help identify unmet needs, and

4. profound understanding of real-world data on treatment decisions and outcomes.

Establishment of a common denominator with a clear ‘value proposition’ needs to be at the core of pricing and re-imbursement submissions. Incremental costs need to consider potential incremental revenues from priority markets (price, positive formulary place, time-to-market) and the potential downside risk of unfavourable comparisons with alternative treatments. IT will in this future play an essential role by assessment of how personalised medicine and prevention will be organised. In many cases, targeted technology will need to be developed, for example intelligent measuring instruments, labs-on-a-chip, nanomedicine and technologies in such areas as genomics, metabolomics and proteomics. Patients, must therefore be involved at an early stage, facilitating both science and enterprise in developing new technological applications based on cost-effectiveness methodologies – known as Health Technology Assessment.

Conclusion

Original new forms of trans-institutional initiatives and teams composed of an unconventional combination of disciplines between university medical centres, universities of technology, social sciences faculties, applied research institutions, and universities of applied sciences, must be extended and broadened in the R&D design and data analysis. The new scientific outlook on prevention, treatment and care should impact the design and financing of research programmes. At times, that may require us to change the rules, for example so that insurers can also invest in research. New publishing paradigms involving risks analysis and technology assessment and new methods for measuring research output can also help drive innovation in the pursuit of science.

References

The most common used antibiotic drugs among dental medicine doctors

Mihajlo Petrovski*, Olivera Terzieva-Petrovska

Faculty of medical sciences, Goce Delcev University, Krste Misirkov 10-a, 2000 Stip, Republic of N. Macedonia

Introduction

Antibiotics are the most commonly used drugs in everyday dental practice. By definition, antibiotics are pharmacological agents that can completely destroy pathogenic microorganisms or prevent their growth or proliferation, without causing significant damage to the host organism-human body. Antibiotics are defined as natural or synthetic organic substances that in low concentrations inhibit or kill selective micro-organisms (Davies & Davies, 2010).

Usually the use of antibiotics in everyday dental practice is characterized by empirical uses based on clinique and bacteriologically commonly known etiological factors. Most dentists use only one antibiotic depending on their experience, but the range of prescribed antibiotics is small and is related to the period, knowledge and experience of dentists (Kaul et al., 2018). However, we are more likely witness of improper use of antibiotics, or using them into treatment where there is no need for them.

The pharmacological properties of antibiotics are crucial and critical in deciding on their use, dosage, ways and frequency of administration. Important pharmacological determinants are body weight, degree of absorption, rate of metabolism, and the duration of effective antimicrobial levels at the site of infection. The efficacy of antibiotic therapy is determined by the antimicrobial spectrum and the pharmacokinetic properties of the drug (Pallasch, 1996).

Therapeutic insufficiencies with some antibiotic medicaments are due to the presence or development of resistant types of microorganisms may be a problem that occurs in dental treatment. For example, the main indication for systemic antimicrobial therapy are those patients who do not result from conventional therapy, or patients with aggressive forms of periodontitis or associated with predisposing medical conditions. Patients with acute or severe periodontal infections (periodontal abscess, acute necrotizing gingivitis or periodontitis) can also benefit from antibiotic therapy (Kapoor et al., 2012).

The main goal of this research was to assess the types and frequency of prescribed antibiotics by dentists, indications for prescribing antibiotics, as well as the knowledge of dentists regarding the use of antibiotics.

Material and method

Nine dental clinics with 14 doctors of dental medicine were included in this study. Eight from the doctors were general dentists, and the other six were dental specialist. Four from the specialist were specialists of oral surgery, one specialist of pedodontics and one for oral pathology and periodontology. Specialists in prosthodontics and orthodontics were excluded from the research.

* mihajlo.petrovski@ugd.edu.mk
The total number of patients for a period of two weeks in which any type dental intervention was performed was registered. An examination was carried out regarding the data on the prescribed antibiotics over two weeks in April 2019.

Dental offices and dentist for the examination were selected randomly examination. Total numbers of ten dental offices were included in this research. During the study, (1) the number of interventions involving antibiotic, (2) the type of antibiotic used for treatment, and (3) the underlying disease due to which antibiotic was involved in the treatment were recorded.

The data obtained from the clinical examination after the collection was statistically processed. For statistical processing was used special software for statistical processing of data- Statistica 7.1.

**Results and discussion**

During the research period, interventions were done on 5764 patients. In 328 or 5.7% cases some type of antibiotic was prescribed. Eighty percent of Most of the examined dentists included in the study prescribed only one type of antibiotic. In only 1.22% in the patients’ population combination of antibiotics was prescribed, such as amoxicillin/clavulonic acid with metronidazole. About 20% of dentists proscribed an antibiotic without performing local treatment.

The most commonly used antibiotics in everyday dental practice in Eastern Macedonia are:
1. Doxycycline - 27.74%
2. Amoxicillin + clavulonic acid - 26.52%
3. Clindamycin - 22.87%
4. Ampicillin - 14.02%;
5. Cefalexin - 8.53%.

The average number of prescribed antibiotics during one week per doctor is 3.24. None of the dentists included in the study have performed appropriate microbiological confirmation for antibiotic selection before the prescription.

According to our study the most common oral diseases where antibiotic treatment has been initiated are: periapical pathologies (most often periapical abscess) - 51.52% or 169 patients, gangrenous teeth in 3.66% or 12 patients, post-extraction wounds and infections 13.41% or 44 patients, the presence of an impacted or semi-implanted third molar teeth in 28.66% or 94 patients and for the treatment of periodontal conditions 2.75% or 9 patients. The most common diagnosis for prescribing an antibiotic is periapical abscess at 44.81%. During the evaluated period none of the dentists initiated antibiotic prescription in prophylactics.

The antibiotic selection should be primarily based on microbiological analyzes from the target zone of the inflammation, but obtained results form everyday dental practice confirmed that the selection was made empirically based on clinical signs.

**Conclusion**

Based on the presented data, it can be noticed that amoxicilinum + clavulic acid and doxycycline are most commonly proscribed antibiotics among dentists. Periapical pathoses was the most common cause antibiotics use in patients in our study. However, the high percentage of prescribed antibiotics indicates the tendency for irrational drug utilization. Our results can contribute in evaluation of rational antibiotics proscription among dentists as well as in developing guideline for rational dental pharmacology practice.

**References**


Legal framework for pharmacovigilance in pregnancy and breastfeeding

Marijana Danevska¹*, Iskra Pechijareva Sadikario¹, Julijana Sekovska², Katerina Ancevska Netkovska³, Zorica Naumovska³

¹Agency for drugs and medical devices, Blvd. Ss. Cyril and Methodius 54, 1000 Skopje, R.N. Macedonia  
²Carso International, St. 34 No 5, 1000 Skopje, R.N. Macedonia  
³Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, R.N. Macedonia

Introduction

Most women undergo medical treatment during pregnancy, irrespectively if the medicine is recommended for a given period or the treatment is unavoidable due to a particular illness. Scientifically based data on the safe use of medicines during pregnancy are an important public health need. In line with this, arise the inevitable need for establishment of pharmacovigilance guidance for the use of medicines during pregnancy on global and national level. The use of medicines during breastfeeding is also an area where further guidance and recommendations are needed. Additionally, important attention should be paid to OTC medicines, especially herbal drugs that are widely used even in the period of pregnancy and breastfeeding and are usually treated as “natural and safe” medicines by the patients.

Spontaneous reporting of adverse reactions is insufficient to routinely detect fetal risks caused by medicine use during pregnancy. Because of limited data on the teratogenic potential of drugs prior to their marketing, post-marketing surveillance of medicine use in pregnancy is of particular importance in detecting medicines-induced fetal risks.

Materials and methods

Relevant European, American and Macedonian legislation was reviewed, in particular, Directive 2010/84/EU, Regulative (EU) 1235/2010, rulebooks, as well as PubMed, Medline and other relevant web sites for articles with empirical analysis, evaluating the impact of European and non-European regulatory activities.

Results and discussion

Since exposure to contraindicated medicines during pregnancy is sometimes unavoidable in each trimester, a safety monitoring mechanism needs to be established to provide reliable information to promote safe and effective treatment during pregnancy. Research involving the treatment of pregnant and breast-feeding women raises ethical and scientific issues. The safety data obtained in the pre-marketing phase of drug development are limited due to the limitations of the clinical trials regarding the size, timing and duration of subsequent monitoring as well as strictly defined inclusion and exclusion criteria of conducted studies (GVP, 2019). Pregnant and breast-feeding women are considered as a special population and are usually excluded from medicine development programs and clinical

* marijana.danevska@malmed.gov.mk
trials, leading to gaps in knowledge when medicines are marketed. It is not known whether effective exposure is achieved during standard-dose pregnancy as in a non-pregnant adult woman and to what extent the medicine crosses the placenta and is excreted in breast milk. Pregnancy is associated with a wide range of physiological, anatomical and biochemical changes that significantly influence the pharmacokinetics of medicines (Pariente et al., 2016). Gathering data to gain a better understanding of the risks associated with such use and identifying and characterizing the risks is important even when no safety concerns have arisen before the pre-marketing phase. Timely and appropriate data collection and assessment enables patients and healthcare professionals to receive relevant information in a timely manner in order to make appropriate decisions about medicine use during pregnancy. The guiding principle is to minimize adverse reactions associated with medicine use during pregnancy.

Successful recruitment of an adequate number of pregnancies exposed to medicines, their comprehensive monitoring and complete and accurate determination of pregnancy outcomes are key elements in creating a well-designed registry or database. Accordingly, the aim is to build capacity for improved maternal and neonatal care within the overall health care system (Gliklich et al., 2018)

This mechanism will help overcome the deficiency commonly seen in medical practice when treating a pregnant woman with ineffective medicines due to potential safety concerns. It can also help assess medicines that are not recommended during pregnancy and which are sometimes unavoidable to save the mother or unborn child. Hence the need to create an appropriate pharmacovigilance guide during pregnancy.

Conclusion

Limited information for drug utilization during pregnancy and breastfeeding rice the need of implementation of additional legal framework in order to obtain safe drug treatment in this special population with positive benefit/risk ratio for the mother and unborn child.

These legal documents in the national level should be harmonized with regulation and rulebooks for good pharmacovigilance practice in pregnancy and breastfeeding already approved and implemented in by EMA.

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The legal and regulatory framework for vaccine pharmacovigilance

Ankica Gestakovska1*, Brankica Moskova2, Zoran Sterjev3, Aleksandra Grozdanova3, Ljubica Suturkova3, Aleksandra Kapedanovska Nestorovska3, Zorica Naumovska3

1Quality Control Department, Replek Farm LTD, Kozle 155, 1000 Skopje, N. Macedonia
2Drug Safety Associate IQVIA, 11 Oktomvri, 7500 Prilep, N. Macedonia
3Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, N. Macedonia

Introduction

Vaccine Pharmacovigilance is defined by the CIOMS/WHO Vaccine Pharmacovigilance Working Group as the science and activities related to detecting, evaluating, understanding and communicating adverse events following immunization (AEFI) and other issues related to vaccines or immunization and preventing vaccination or vaccine side effects (CIOMS, 2018).

Well established pharmacovigilance system enables early detection, an appropriate and timely response to AEFIs, minimization of the negative effects on the public health, and reduction of the potential negative impact on immunization in the population. Continuous risk-benefit assessment and risk management are an integral part of the pharmacovigilance monitoring of the vaccines. The legal framework for vaccine surveillance during the clinical phase of vaccine development and post-marketing period is essential for the implementation of good pharmacovigilance practice on a global and national level. This system should empower the public trust in the healthcare system to obtain sufficient vaccination especially for preventable diseases and improve public health nationally and globally (Di Pasquale, 2016; EMA, GVP, 2013).

Materials and methods

Relevant European, American and Macedonian legislation was reviewed, in particular, Directive 2010/84/EU, Regulative (EU) 1235/2010, rulebooks, as well as PubMed, Medline and other relevant web sites for articles with empirical analysis, evaluating the impact of European and non-European regulatory activities.

Results and discussion

The vaccine supplying process is established and regulated system by national competent authorities and regularly assessed by WHO. In the process of pharmacovigilance system assessment and AEFI monitoring, seven indicators are defined and six of them are predominantly important. Licensing, marketing authorization, and pharmacovigilance of vaccines are mandatory for all countries, irrespectively if they produce vaccines or not. Also, the WHO recommends that all countries that do not produce vaccines, however, must define minimum specifications for the vaccines they use. A system of post-marketing surveillance should be established to detect vaccine efficacy or safety problems. In all countries, AEFIs should be monitored, reported and investigated by national competent authorities. Also, NCA must create a user-friendly and appropriate...
monitoring system of AEFIs. Additionally, a close and clear communication and information exchange system should be established among the NCA and all concerned stakeholders (Fulton, 2015; Global vaccine safety initiative, WHO 2018).

Marketing authorization, distribution, and vaccines pharmacovigilance are strictly regulated in the Republic of North Macedonia. In line with EU legislation, the national competent authority MALMED enables the controlled release of each vaccine lot after certificate assessment and inspection supervision. It has the main role in ensuring the implementation of good distribution practice procedures. An effective immunization surveillance system requires the involvement of health professionals at all levels of the immunization program. Detecting and reporting of AEFI is a responsibility of all healthcare professionals in outpatients, clinics and vaccination sites. Health personnel must have adequate training for providing proper immunization, detecting, and reporting of potential AEFIs (EMA, GVP, 2013; Mehta et al., 2000).

All detected AEFI in the Republic of North Macedonia should be reported to MALMED and the Institute of public health. Since 2018, the Republic of North Macedonia has established an electronic system for reporting adverse drug/vaccine events to enable better accessibility for health care professionals (HCPs) and patients. All collected AEFI data has a strictly defined timeline submission to the Uppsala Monitoring Center (UMC) in E2BR3 format, depending on the seriousness of the event. All reported AEFIs are assessed on the national level. A proper communication system is established for all stakeholders to increase the early detection of signals and the implementation of risk minimization or corrective measures. Only 5 AEFI cases were reported in 2017, 7 in 2018 and all of them were expected non-serious reactions. In 2019, during the measles epidemic in the Republic of North Macedonia, over 42000 MRP vaccines were given to the population. The vaccination process ended up with only 17 AEFI reported, among which only 11 were associated with the MRP vaccine. All the reported AEFI were non-serious expected events according to information listed in approved vaccines SmPCs.

As we evaluate the available data from the competent authorities, it could be noticed that the Republic of North Macedonia is facing a big problem in reporting ADRs as well as AEFIs. The small number of reported events doesn’t confirm their absence. Uncontrary, it points out that an additional educational program should be initiated to train the HCPs for detection, reporting, and evaluation of adverse events and raise the awareness for the importance of good pharmacovigilance system in our country.

Conclusion

Establishing an appropriate legal and regulatory framework and complete harmonization of national regulations with EU legislation for vaccine pharmacovigilance is inevitable. This should be sound ground for improved continuous monitoring of vaccine efficacy and safety, as well as identification of potential safety signals, conduction of risk minimization measures and better public health in the Republic of North Macedonia.

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Legal framework for use of social media in pharmacovigilance

Brankica Moskova1*, Ankica Gestakovska2, Zoran Sterjev3, Aleksandra Grozdanova3, Ljubica Suturkova3, Aleksandra Kapedanovska Nestorovska3, Zorica Naumovska3

1Drug Safety Analyst IQVIA project member, 11 Oktomvri, 7500 Prilep, N. Macedonia
2Quality Control Department, Replek Farm, Kozle 188, 1000 Skopje, N. Macedonia
3Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, N. Macedonia

Introduction

In accordance with the ICH E2D Guidelines, marketing authorization holders (MAH) are obliged to facilitate the collection of data on potential reports of suspected adverse reactions through their websites (for ex by providing direct reporting templates or through tools that enable direct communication). If MAH receives information about a suspected adverse reaction originating from a digital media sponsored by other companies, they should individually assess whether that report is appropriate to be submitted as an Individual Case Safety Report-ISCAR. Suspected adverse reactions received through websites or digital media sites are considered as spontaneous reports (Regulation (EU) No 520/2012).

Many companies on their own social media sites (Twitter, LinkedIn, etc.) to disseminate information about the company's products. In modern society social media could be used as potential tool in post-marketing drug safety monitoring and this approach is slowly but surely accepted by the pharmaceutical industry. For example, some companies use social networking sites to monitor health forums and share information between patients and healthcare professionals about specific pharmaceuticals or health conditions.

Materials and methods

Relevant European legislation was reviewed, in particular, Regulation (EU) No 520/2012, ICH E2D harmonized tripartite, EU (The Innovative Medicines Initiative Web-Recognizing Adverse Drug Reactions- IMI WEB-RADR) project enrolled in 182 countries, as well as PubMed, Medline and other relevant web sites for articles with empirical analysis, evaluating the impact of European and non-European regulatory activities (Brosch et al., 2019; Ghosh et al., 2019; Regulation (EU) No 520/2012).

Results and discussion

The modern approach to tracking adverse drug reactions employs the Natural Language Program (NLP), which uses data for medicines and associated adverse reactions located on social media. Information posted on social media by drug users contains information on treatment outcomes and provides information that can be used to create reports of adverse reactions. These information are available although no valid reports for adverse drug reactions have been made and have crucial importance for all healthcare professionals and the pharmaceutical industry (Duh et al., 2017).

The type and volume of information for adverse drug reactions made available through the social

* brankica.moskova1977@gmail.com
media to the pharmaceutical/healthcare industry cannot be easily provided in any other way. These include adverse reactions occurring in specific patient populations such as patients with rare diseases, pregnant women, nursing mothers, geriatric and pediatric populations or patients with comorbidities, which are usually excluded from clinical trials. There are still many questions that need to be answered concerning the ability of pharmaceutical companies to implement social media screening systems. One possibility for pharmaceutical companies is to establish patient support groups through social networking platforms to provide and share all relevant information about their products. These platforms can also carry out a series of activities that are part of risk minimization measures that sometimes only apply to healthcare professionals, but sometimes include patients as well. New tools and data sources, such as social media, provide numerous opportunities for enhancing public health, including opportunities to collect safety information, helping to generate new insights into drug benefit/risk profile, as well as unique insights into pharmacovigilance system development, in general. It is crucial that these opportunities are utilized ethically and in accordance with accepted regulations, in order to respect the privacy of data and to use it responsibly (Limaye and Saraogi, 2018). All healthcare professionals (doctors, nurses, dentists, and pharmacists) play a pivotal role in prescribing, dispensing and administering of the drugs, but they also have an essential role in monitoring of treatment outcome, identifying and reporting of adverse events in post-marketing period, providing an efficient pharmacovigilance system. Social media can afford a serious platform for enhancing the connection between healthcare workers and their patients, providing a basis for sharing credible information about medications and therapeutic opportunities. Patients often engage in discussions with healthcare professionals on these sites and share their personal experiences on a specific drug, which can be a good basis for timely detection of new potential drug safety signals resulting in taking suitable actions for enabling efficient and safe use of the medicines available on the market (Caster et al., 2018).

There are few limitations in implementation in everyday pharmacovigilance practice. Medicines on social networks can be listed in a non-standard way, under their trademarks, generic names, or the active ingredients they contain.

The listed adverse events may be represented by idiomatic expressions that do not exist in medical lexicons and dictionaries. Informal communication on social media is an additional obstacle as bad grammar, shortcuts or slang are often used.

Conclusion

After establishment of appropriate legal framework and program languages the social media can be successfully used as effective source of information in modern pharmacovigilance practice worldwide. The risk-based approach applied aims to make better use of these new tools and data sources. The objectives of the developed principles are to guarantee continuous monitoring of drug safety and timely identification of potential safety signals without overloading pharmacovigilance systems.

References


Risk management system and risk minimization measures as crucial part in implementation of good pharmacovigilance practice

Marija Guleva¹*, Angela Mircheska-Janevska², Zoran Sterjev³, Aleksandra Grozdanova³, Ljubica Suturkova³, Aleksandra Kapedanovska Nestorovska³, Zorica Naumovska³

¹Farma Trejd dooel, Anton Popov street No. 1/1-3, 1000 Skopje, N. Macedonia
²Nostrapharm dooel, Slave Delovski street No. 7, 1000 Skopje, N. Macedonia
³Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, N. Macedonia

Introduction

Each drug is authorized for a specific indication(s) based on a positive risk-benefit ratio confirmed in the clinical phase of drug development. It is generally expected in post marketing period each drug to be associated with adverse reactions that vary in severity, likelihood of occurrence, effect on different patients, and impact on public health. All adverse reactions and risks may not be identified during the initial application for marketing authorization and some will be identified and characterized in the post marketing period.

In EMA the pharmaceutical companies have to submit risk management plan while applying for marketing authorization. In addition, for medicines registered by national procedure, national competent authorities in EU may request RMP to be submitted whenever there is a suspicion of a benefit-risk balance of the drug. The RMP is a dynamic document that is continuously modified and updated over the drug life cycle as more information on the drug's safety profile is obtained that influence its safety profile (GVP, Risk management system, 2017). FDA has had many regulatory initiatives that could be classified as forms of risk management procedures, such as classification of some OTC drugs in prescription drugs, since it ensures their safe use only under supervision of a HCPs. Prior to 2007, the pharmaceutical companies in the United States have been submitting specific safety programs: Risk Management Programs (RMPs) or Risk Minimization Action Plans (RiskMAPs) for a limited number of drugs with significant therapeutic benefit, while starting from 2007 the companies are submitting Risk Evaluation and Mitigation Strategy (REMS) (FDA, 2018).

Materials and Methods

Relevant European, US and Macedonian legislations have been reviewed, in particular, Directive 2010/84/EU, Regulative (EU) 1235/2010, rulebooks, as well as PubMed, Medline and other relevant web sites for articles with empirical analysis, are evaluating the impact of European and non-European regulatory activities.

Results and Discussion

According to EMA and FDA legislation MAHs should have established appropriate risk management system and should continuously monitor the safety profile of the medicinal product by monitoring the pharmacovigilance data in order to determine whether there are new risks, or changes

* gulevamarija.ft@gmail.com
in the risks or risk-benefit ratio of the drugs in order to update the risk management system and RMP accordingly. The safety profile of the medicinal product should be constantly monitored and presented in the updated Periodic Safety Reports (PSUR). Although PSUR is a retrospective, integrated post-marketing document and RMP is prospective pre- or post-marketing document, both are complementary documents (GVP, Risk management system, 2017).

The RMP consists information for the drug’s safety profile, information on how the risks associated with the drug use will be prevented or minimized in patients, study plans and other activities to provide more information on the safety and efficacy of the drug, and to determine the effectiveness of the implemented risk minimization measures (GVP, Risk management system, 2017).

On the other hand, REMS consists information that should be transmitted and / or required activities to be taken by healthcare professionals, pharmacists, patients who prescribe, dispense or use medication. Together, these activities provide a secure strategy in effective, safe and rational drug use (FDA, 2018).

In some cases, both documents, RMP and REMS, include additional requirements such as clinical activities that HCPs may need to perform prior to prescribe or dispense the drug to the patient. Such example is severe allergic reactions immediately after administration of the drug, so risk minimization measures are needed to ensure that the drug is administered only in healthcare facilities with HCPs trained to deal with severe allergic reactions or the need of lab testing and checking of results before administration of the drug. Risk minimization measures may include education of HCPs for certain patient groups that are more likely to experience an adverse event and thus avoiding prescribing the drug in these patients, or the need for patients cards with important information for the drug (FDA, 2018; GVP, Risk management system, 2017).

Risk minimization strategies should be well evaluated from the time of drug development. The knowledge of the constituents or class of the drug in the period of drug development is sufficient for prediction of the post-marketing risk minimization activities. The use of these strategies can significantly reduce the post-marketing risk of the drug (Dieck and Sharrar, 2013; Mollah, et al., 2014).

There are number of risk minimization activities that can be introduced, starting from packaging design, examination of educational material, to more complex activities such as safety studies. These pre-marketing activities will not help to avoid the occurrence of unknown, serious risks identified in the post-marketing period, but could help the manufacturer to responds quickly and effectively to unexpected safety-related events and to properly manage the identified risks (Dieck and Sharrar, 2013).

Conclusion

In N. Macedonia all risk minimization measures are approved by MALMED through the Committee for safety and advertising since the current Law does not contain sufficient information regarding the risk management. These local legal documents should be harmonized with regulation and rulebooks for good pharmacovigilance practice approved and implemented by EMA.

References


Cost-effectiveness of combination LMWHs/UFH versus UFH/LMWHs for the prevention of postsurgical venous thromboembolism at orthopedic department in Clinical Hospital Stip

Biljana Lazarova¹*, Zorica Naumovska², Aleksandra Kapedanovska Nestorovska², Zoran Sterjev², Ljubica Suturkova²

¹Clinical hospital, Ljuben Ivanov bb, 2000 Stip, N. Macedonia
²Faculty of Pharmacy, Ss Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, N. Macedonia

Introduction

Venous thromboembolism (VTE), which includes both deep vein thrombosis (DVT) and pulmonary embolism (PE), is a major burden on the health care system. The risk of VTE is particularly high in patients who undergo major orthopedic surgical interventions, especially interventions for total hip or knee replacement due to perioperative activation of blood coagulation, the effects of surgical trauma of the femoral and iliac vein or embolism due to prolonged bed stay (Imberti et al., 2011). Consequences of VTE and its long-term complications can significantly impair the quality of life in terms of patient health, while the treatment of the condition and recurrent complications become significant costs for the health care provider. Costs are also made during the period of hospitalization immediately after surgical interventions for total hip replacement (THR) and total knee replacement (TKR), as well as in months after discharge from the hospital. Thromboprophylaxis significantly reduces the risk of perioperative VTE. The longer duration of thromboprophylaxis, the lower incidence of VTE. Without anticoagulant prophylaxis, about 50% of patients with symptomatic proximal DVT or PE have a recurrent thrombosis within three months (Torbicki et al., 2008). The most frequently recommended VTE prophylaxis in the 2004 ACCP consensus guidelines is lowmolecular-weight heparin (LMWH) or unfractionated heparin (UFH).

The updated recommendations in 2008, released by ACCP include fondaparinux alongside LMWH and UFH for the prevention of VTE in certain patient populations (Geerts et al., 2008). There is no scientifically proven evidence of thromboprophylaxis that begins with UFH and continues with LMWHs and vice versa. During the study period in orthopedic ward 40 patients were identified where thromboprophylaxis was started with one drug and then continued with the other. Having that in consideration we evaluated the cost effectiveness of LMWHs/UFH compared to UFH/LMWHs for the prevention of VTE in this particular patients.

Materials and methods

In cost-effectiveness analysis the costs are reported in MKD values, and health outcomes are
converted into Quality Adjusted Life Years (QALYs), incorporating the measure of quality of life (utility) in health outcomes. Cost-effectiveness between LMWHs/UFH vs. UFH/LMWHs used as thromboprophylaxis in patients underwent orthopedic surgery hospitalized at the orthopedic department in Clinical Hospital Stip was revised. The decision for thromboprophylaxis regime was made by the surgeon’s, dependent on the availability of anticoagulants at the hospital pharmacy.

**Decision tree model**

A decision tree model was developed (Tree Age Pro 2013 software, Williamstown, MA) in order to evaluate the cost-effectiveness and results of prophylaxis of DVT with LMWHs/UFH vs UFH/LMWHs in high-risk patients who underwent orthopedic surgery.

**Results and discussion**

In the decision tree model analysis, the application of thromboprophylaxis with UFH/LMWHs or LMWHs/UFH combinations were evaluated. The first branching in decision tree has two branches: thromboprophylaxis with LMWHs/UFH, costing 60854.88 MKD and 9.84 QALY per patient, whereas thromboprophylaxis with UFH/LMWHs cost 85605.99 MKD and 70 QALY per patient during hospitalization. Each of the branches was further divided into 4 new branches showing the probability of occurrence or non-occurrence of VTE (DVT and PE). In patients undergoing thromboprophylaxis with the combination LMWHs/UFH the probability of stable condition in patients hospitalized for up to 11 days was 0.991. The probability of occurrence of DVT is 0.00 and the incidence of PE was 0.009.

In patients undergoing thromboprophylaxis with a combination of UFH/LMWHs, the probability of stable condition for up to 11 days of hospitalization was 0.915, and the probability of developing DVT was 0.085, for PE the probability was 0.00. Decision tree analysis of combined thromboprophylaxis showed higher cost-effectiveness of LMWHs/UFHs with lower cost, greater efficacy and likelihood of a stable condition being greater than UFH/LMWHs. The results for combined thromboprophylaxis over a 6-month period showed greater dominance of the LMWHs/UFH combination with an effectiveness of 5.01 QALYs and cost of 30884.76 MKD compared to a combination of UFH/LMWHs where a cost effectiveness of 4.70 QALY is less than 42707Y. ICER is -137204.69 MKD/QALYs. Combined thromboprophylaxis results evaluated in period over a 12-month presented even greater dominance of the LMWHs/UFH combination with an effectiveness of 9.84 QALYs at a cost of 60854.88 MKD compared to a combination of UFH/LMWHs where a greater cost of 85605 of 9.70 QALYs. ICER is -174939.72 MKD/QALYs. The increased cost is 24751.11 MKD and the increased effectiveness is -0.14 QALYs.

**Conclusion**

Cost-effectiveness analysis confirmed that LMWHs dominated vs. UFH in major orthopedic surgery, providing greater effectiveness at lower costs. LMWHs also lead to less symptomatic VTE events providing increased QALYs comparing to UFH. The use of LMWHs in this prophylactic indication contributes to the effective use of limited resources, as it is associated with better clinical results at a lower cost.

**References**


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Evaluation of antibiotic consumption in Clinical hospital in Stip for the period 2017-2019

Biljana Lazarova1*, Biljana Eftimova1, Lidija Mihailova1, Sanja Filkova2, Zorica Naumovska3, Zoran Sterjev3

1Clinical hospital, Ljuben Ivanov bb, 2000 Stip, North Macedonia
2Department of Clinical Pharmacy, Mother Theresa 17, 1000 Skopje, North Macedonia
3Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, North Macedonia

Introduction

Antibiotics are widely used in clinical practice globally and their irrational use could lead to antimicrobial resistance and negative therapeutic outcome even in a case of easy to treat diseases. The misuse and overuse of antibiotics additionally initiate negative effects on the normal bacterial flora in patients, triggering superinfections, adverse event occurrence and subsequently increase of treatment costs. One of the most important fact for irrational antibiotic utilization is the negative impact on the hospital microbial environment erasing the inevitable need for permanent monitoring of the spread of bacterial resistance in everyday clinical practice (Cizman et al., 2003). Nowadays healthcare professionals face serious problem while infections caused by resistant microorganisms gradually increase, antimicrobial options diminish and global market lacks new antibiotics. Monitoring the antibiotic consumption and prescription pattern are critical due to the fact that they could provide valuable data for adherence to globally accepted guidelines in clinical practice.

The aim of this study is to evaluate antibiotic consumption in the Clinical hospital in Stip, with the ATC/DDD index, which is an accepted standard method in three years period 2017-2019.

Materials and methods

A retrospective study was carried out at the Clinical hospital in Stip, in order to evaluate the antibiotic consumption in period 2017-2019. The data were obtained for the following ten departments (internal medicine, neurology, infectious diseases, general surgery, orthopedics, urology, gynecology, obstetrics, pediatrics, intensive care unit) with total of 288 beds. Antibiotics utilization was evaluated based on dispensed antibiotics with ATC code J01 (regardless of the indication for which they were used - prophylaxis or therapy) for each department provided from the hospital pharmacy. The ATC/DDD index is a universal parameter used in the evaluation of antibiotic use (Filius et al., 2005).

Total number of hospital days (bed/day) BD, during a certain period of time and the index of occupancy of hospital beds were collected from statistics department in the Clinic. The DDD is the assumed average maintenance dose per day for a drug used for its main indication in adults. The quantity of antibiotics was converted into a number...
of defined daily doses (DDD/100 bed-days (DBD)) via the anatomical-therapeutic-chemical (ATC) and DDD drug classification. DDD values of every antibiotic is calculated separately with Antibiotic consumption calculator (ABC Cal Version 3.1 constructed by Monnet DL, Staten Serum Institute 2006. DDDs = Number of boxes x number of tablets in the box or number of vials x grams of active compound in tablet or vial/the DDD value of the antibiotic in grams. In this calculation method, the form used for in-bed patients is the ratio of the total DDD per 100-bed-days. This index is called antimicrobial consumption index (ACI). ACI=DDDs/bed–days×100 (Kuster et al., 2008; WHO, 2010).

Results and discussion

In our study, cephalosporins were found to be the most frequently used antimicrobials in all evaluated departments with the highest consumption of 3rd generation cephalosporin, ceftriaxone. The highest utilization of ceftriaxone with over 94.5% and i=0.50 was confirmed in the departments for gynecology. On the internal medicine department, ceftriaxone accounts 82% with i=0.89 in 2017; 71.4%; i=0.79 in 2018 and 41.59% with i=0.59 in 2019. This declination observed for ceftriaxone, resulted in increased consumption of ciprofloxacin with 37.2% compared to year 2018 (14.05%) and 2017 (12.6%). The same pattern in evaluated three year period was confirmed, in the neurology, pediatrics, infective and urology departments, with increased utilization of fluoroquinolones. On the neurology department, ceftriaxone accounts for 94.4% with i=0.79 in 2017, 94.7%; i=0.62 in 2018 and 85.99% with i=0.62 in 2019. The increase of fluoroquinolone ciprofloxacin was also confirmed on this department with account of 2.43%, 3.82% and 7.54% for 2017, 2018 and 2019 respectively. Increase from 8.07% to 12.94% was observed in the department for infectious diseases for ciprofloxacin and decrease from 83.78% to 76.71% with i=0.52 for cephalosporin from 2017 to 2019. Metronidazole was the second most frequently utilized anti-infective with 10.63% to 12.22% and i=0.04 for the evaluated period in the intensive care unit. Significant decrease of clindamycin consumption was confirmed in the general surgery department in evaluated period and increase of metronidazole use in 2017, 5.99% and i=0.39 compared to 2019 with 9.64% and i=0.54. Same pattern was confirmed in orthopedic department in three year period. The frequent use of cephalosporins and fluoroquinolones lead to the emergence of resistant microorganisms, thus problems such as the emergence of resistant pathogenes in our area would be an inevitable consequence. As antibiotics are the most frequently used drugs in the hospitals they constitute an important part of the total drug expenditure.

Conclusion

Utilization of ATC/DDD system in hospitals can provide internationally valid data in the evaluation of antimicrobial use. These data should result in more efficient antibiotics utilization in hospitals, and decisions made in line with the recommendations in established guidelines. Reducing irrational antibiotics use would prevent the incidence of life-threatening serious infections, also preventing long hospital stays and higher health expenditures.

References


Patient registries in regulatory decision making  
- a survey of Macedonian registries

Elona Chilku¹*, Goran Kochinski², Kristina Mladenovska³

¹Agency for Medicines and Medical Devices, Cyril and Methodius 54, 1000 Skopje, N. Macedonia
²Ministry of Health, e-Health Directorate, 50 Divizija 14-a, 1000 Skopje, N. Macedonia
³Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, N. Macedonia

Introduction

Patient registries are “organized systems that use observational methods to collect uniform data on a population defined by a particular disease, condition, or exposure, and that is followed over time” (EMA, 2017). According to ISPOR, a registry includes a “prospective observational study of subjects with certain shared characteristics, which collects ongoing and supporting data over time on well-defined outcomes of interest for analysis and reporting” (Polygenis et al., 2013). The data collected help policy makers, researchers, healthcare professionals and many others to prioritize their activities and actions and plan restricted resources in areas of their responsibilities. Establishing and governing a patient registry requires use of standardized methodologies, processes and technologies that will enable researchers, health technology assessment organizations and policy creators to compare, analyze and use data from different (inter)national registries for extracting new knowledge and make informed decisions about individual patients and or entire populations. In this paper, the results from the survey of patient registries established and planned in the Republic of N. Macedonia (RoM) are presented. In addition, an overview of funding and legislation concerning registry set-up, sustainability, data protection and re-use is given.

Materials and Methods

To collect the information on national patient registries, a questionnaire was used, consisted of questions related to the primary propose of the registry, data collection, handling, funding and governing. Some of the stakeholders had been interviewed in person or by phone. The target groups were government institutions, academia, clinical centers and hospitals, professional and patient associations, and marketing authorization holders.

Results and Discussion

Thirty-five registries were foreseen at national level. Out of them, 22 are disease/condition based (registries of patients having the same diagnosis or the same group of conditions), 10 are health service based registries (consisted of patients having a common procedure, clinical encounter or hospitalization) and one is registry based on product (patients exposed to biopharmaceutical products, medical devices or diagnostic/therapeutic equipment), while 3 are not patient registries, but registries of healthcare professionals and institutions. In disease/condition category several subcategories

* elonachi@gmail.com
have been recognized based on organ system or clinical field, with the largest number (4) falling under the cancer/tumor (integrated in one Cancer Registry) and injuries/accidents subcategories, followed by infectious (3), coronary/vascular (2), congenital (2), substance abuse/addiction (2), renal/urogenital (1), disabilities (1), mental/psychiatric (1) and diabetes/metabolic/endocrine (1) subcategories. Rare diseases are incorporated in one register only.

The service-based patient registries are divided into the following subcategories: obstetric and gynecological services (births, abortions, medically assisted fertilization) (3), preventative services, quality of care and health monitoring (3), registries of donors (blood and organ) (2) and causes of deaths (2). The Insulin Registry is the one based on product.

Out of 35 foreseen patient registries, 10 registries are already established and active in electronic form, 3 are expected to be activated during 2020/2021 and 10 are in process of preparation. Comparing to the active registers across the European countries (data from 2015), the number of active registers is lower than the one in Austria and Italy (38 each), Finland, Sweden, Croatia, Poland, Norway and Slovenia (between 32 and 15), but higher than in the neighboring countries, Greece and Serbia (two each) and Albania and Bulgaria (one each) (Zaletel and Kralj, 2015).

The registries in the RoM are dominantly governed by the Ministry of Health. They contain information from all institutions in which patients are diagnosed and treated for the corresponding disease or the healthcare services are provided. Thus, all national registries are funded by national government authority and there is “no specific funding” such as an umbrella organization, certain project, etc. The Institute of Public Health uses these data for health care planning, incl. primary prevention, diagnosis and treatments. Because of the limitations of premarking clinical trials (short duration, small sample size, narrowly defined population, limited comparison groups, etc.), observation of medical products after their approval for marketing is important, especially for products granted conditional marketing authorization, since registries may provide data to confirm their safety and/or effectiveness. However, the registries in RoM are still not extensively used for benefit/risk evaluation of medicines.

Most of the registries are based on electronic health care records, although paper based questionnaires/health records/laboratory results are still used for data collection. As literature data point out, almost half of the EU registries are still based on paper-and-pen mode, which “causes lower data quality, it is costly and time consuming and does not allow any control of the data filled in” (Zaletel and Kralj, 2015). The set-up, sustainability, data protection and re-use of patient registries are regulated by the Law on medical records (Official Gazette of RM 20/09) and the Rulebook on how to access, distribute, publish, use, store and protect data from the integrated health information system (http://zdravstvo.gov.mk). Insufficient information on quality standards and no quality control/assessment tools for the registries in the RoM was available. The data contained in the registries are available to researchers from other institutions and there are protocols that enable them to access the data.

**Conclusion**

The number and subcategories of patient registries in the RoM comparing to the European countries is satisfactory. However, awareness of quality standards should be increased and quality assessment tools introduced. As patient registers are opened for research purposes, their extensive utilization for post-marketing evaluation of medicines should be also considered.

**References**


Patients’ satisfaction and the pharmacist’s role in hospital settings

Sanja Filkova\textsuperscript{1}\*, Olivera Krsctic Nakovska\textsuperscript{2}, Sava Pejkovska\textsuperscript{2}, Dimitar Karkinski\textsuperscript{2}, Jasminka Patcheva\textsuperscript{3}

\textsuperscript{1}Public institution for the needs of University Clinics, Institutes and Urgent Centre, Department of Clinical Pharmacy, Majka Tereza 17, 1000 Skopje, Republic of North Macedonia

\textsuperscript{2}University Clinic of Pulmology and Allergology, Majka Tereza 17, 1000 Skopje, Republic of North Macedonia

\textsuperscript{3}Pharmaceutical Chamber of Macedonia, 50 Divizija 34, 1000 Skopje, Republic of North Macedonia

Introduction

Clinical pharmacists are specially trained practitioners who provide direct patient care and comprehensive medication management (Jacobi, 2016). Since the medication management is the primary focus, the role of the pharmacist obtains optimal use of medications and avoidance of the adverse effects through pharmacist’s education, monitoring and intervention (Jacobi, 2016). Since the traditional role of the pharmacist in the procurement, dispensing, manufacturing and supplying of the medicines evolved in pharmacist’s care service provider, the center of the pharmacist’s main activities beside the therapy became the patient (Onatade et al., 2018). The clinical services of the pharmacist include ensuring that there is an appropriate indication for each medicine, selecting and recommending the most appropriate medicine and dose regimen, providing medicine, ensuring the appropriate administration, monitoring drug therapy, counseling patients and evaluating effectiveness (Webb et al., 2015). The patient’s compliance to the therapy and the outcomes of the treatment deeply depends of the patient’s satisfaction of provided services basically on patient’s perspective of quality and effective service (Grady and Reichert, 2014). Measuring patient’s satisfaction with the pharmacist’s services they receive is imperative for the purpose of their successful implementation, long term sustainability and quality improvement in health care delivery (Shelton, 2000).

Aim and objectives

Clinical services provided by pharmacist at the University Clinic of Pulmology and Allergology in Skopje, Republic of North Macedonia were not introduced until 2018 because there was not employed pharmacist. In June 2018 the pharmacist with the relevant education, competences and skills started to work and the clinical services started to be implemented. One of the services that pharmacist started to provide was counseling the patients with chronic obstructive pulmonary disease (COPD) and asthma for the proper use of the medications. The idea for evaluating patients’ satisfaction came as a result that we haven’t found any satisfaction survey that had been performed before on the clinical services provided by the pharmacists working in hospital settings. The objective was to evaluate patients’ satisfaction with clinical pharmacist’s services and the pharmacist herself. It was important to find if there were differences in patient
satisfaction, in the provision of the service consultation on pharmacotherapy, in understanding the use of medicines and the adherence of the therapy after the engagement of the pharmacist in the clinic since the interviewed patients were all chronic ones that have visited the clinic for several years before and after the employment of the pharmacist.

Materials and methods

An anonymous patient satisfaction survey was offered to patients receiving COPD and asthma treatment at the University Clinic of Pulmology and Allergology in Skopje, Republic of North Macedonia. It contained demographic data like gender, age, education level and profession, 4 statements that patient evaluated according Likert psychometric scale, ranging from totally disagree to totally agree and 7 open-ended questions. The statements were used for assessing the patient satisfaction from the quality of the service, whether the service had improved in the presence of the pharmacist, the need of increased use of pharmacist’s services in the future and the need of implementing pharmacist’s clinical activities in other hospital settings. The opened-ended questions were intended to provide the data whether the pharmacists had an appropriate conversation with the patient about pharmacotherapy, if the provided information by the pharmacist was understandable and useful, if the patient clearly understood the role of the pharmacist and what should be done for the improvement of the provision of the services. Criteria for participant inclusion consisted of chronic patients that have visited the clinic in the several past years, 2 – 3 times a year, male and female. The survey was performed from September to December 2019.

Results and discussion

A total of 64 patients participated in this study, 28 females and 36 males. Patients were between the ages of 43 and 84. The results have shown that total patients’ satisfaction for the quality of the clinical services provided by the pharmacist is better than average (4.1), and almost all fully agreed that the services are improved in the presence of the pharmacist (4.8). Most respondents rated high the need for increasing the rate of provided services by the pharmacist (4.4) and the need of receiving such services in other hospital settings (4.8). There was no significant difference in provided answers based on demographic factors, level of education and profession with patient satisfaction.

Conclusion

This study has shown that the activities and the role of the pharmacist were fully recognized by the patients. The evaluation of patient satisfaction with pharmacy service may be a valuable means of feedback for the pharmacists providing clinical services, for the hospital and clinical settings, mainly for evaluating the quality of provided care in order to improve patient treatment and outcomes. Performing patient satisfaction studies should be more frequent and more standardized in the future in order to improve the provision of the services.

References

Patients’ knowledge and awareness of herbal medicines efficacy and safety in hospital settings

Sanja Filkova¹*, Branka Pashaliska Cvetkov², Jasminka Patcheva³, Sava Pejkovska⁴, Olivera Krstic Nakovska⁴, Dimitar Karkinski⁴

¹Public institution for the needs of University Clinics, Institutes and Urgent Centre, Department of Clinical Pharmacy, Majka Tereza 17, 1000 Skopje, Republic of North Macedonia
²University Clinic of Neurology, Majka Tereza 17, 1000 Skopje, Republic of North Macedonia
³Pharmaceutical Chamber of Macedonia, 50 Divizija 34, 1000 Skopje, Republic of North Macedonia
⁴University Clinic of Pulmology and Allergology, Majka Tereza 17, 1000 Skopje, Republic of North Macedonia

Introduction

Herbal medicines are one type of dietary supplements and people use them to try to maintain or improve their health and well-being. Many people believe that products labeled “natural” are always safe and good for them. This is not necessarily true. Herbal medicine probably presents a greater risk of adverse effects and interactions than any other complementary therapy (Vickers et al., 2001). Some dietary supplements have the potential for substantial hazard. These hazards raise issues about the regulatory and practical distinction between dietary supplements and drugs (Palmer et al., 2003). In addition, there is an ongoing problem with unexpected toxicity of herbal products due to quality issues, including use of poor quality herbal material, incorrect or misidentified herbs, incorrect processing methods, supply of adulterated or contaminated herbs or products (Shaw, 2010). Adverse reactions due to interactions may not be recognized if the physician or other health professional is not aware of the concomitant use of medicinal herbs (Giveon et al., 2004). Increased symptom severity was associated with use of several ingredients, long-term use and age. Associations between adverse events and ingredients are difficult to verify if a product has more than one ingredient, and because of incomplete information systems (Palmer et al., 2003). Vickers et al. (2001) have shown that “Serious adverse effects after administration of herbal products have been reported, and in most cases, the herbs involved were self-prescribed and bought over the counter or were obtained from a source other than a registered practitioner”. Overlap of some pharmacological profiles further blurs the boundary between prescription pharmaceuticals and dietary supplements (Palmer and Howland, 2001). The role of the clinical pharmacist as an integral part of modern health care in hospital settings in terms of use of herbal medicines and dietary supplements is very significant. There are relatively few data regarding the use of herbal products in hospitalized patients and the incidence of such events remains unknown. The aim of this study is to determine the level of use of herbal products and other dietary supplements, evaluate and increase patients’ knowledge and awareness of supplements efficacy and safety, and to explore whether there is a need to document the use of herbal products in the hospital settings.

* s_filkova@hotmail.com
Materials and methods

This survey presents and discusses results obtained from analyzing responses to an anonymous questionnaire collected from patients receiving multiple sclerosis treatment at the University Clinic of Neurology in Skopje, North Macedonia. The questionnaire consisted of 14 questions. It contained demographic data such as gender, age, education level and profession and 10 questions related to the patients’ use and knowledge of herbal medicines and exchanging information with medical staff about them. The survey was performed from December 2019 to February 2020. The obtained data were documented and descriptively analyzed.

Results and discussion

A survey was conducted among 36 patients with multiple sclerosis, 16 females and 20 males. The patients were between the ages of 38 and 73 years and they come from various North Macedonian cities. 72% of the respondents used herbal medicines and other dietary supplements before hospitalization; 44% reported to have either enough or a lot of knowledge of dietary supplements, while 56% knew little or nothing. Most often, they used Vitamin D3, Vitamin B, Vitamin C, Vitamin E, cannabis oil, aloe vera gel, garlic, St John’s wort, valerian and herbal mixture. Family and friends were the main influence that determined use in 77% of the respondents, whereas a physician’s or a pharmacist’s referral was reported in 13%, whereas media account for 7% of the cases. Only 23% of patients considered that herbal medicines can cause side effects, and 27% were aware of the possibility of interactions with conventional drugs. On admission to the hospital, 81% of the patients were not asked by the doctor if they used herbal medicines or other dietary supplements, while 89% of the patients considered that it was necessary to inform the doctor about it. There was no significant difference in provided answers based on demographic factors, level of education and profession with patient knowledge.

Conclusion

These findings confirm that a majority of patients used herbal medicines and vitamins without being asked about it by a physician or without informing him on their own initiative. They are still not aware of the risks to their health, the reasons for missing therapeutic efficacy, adverse effects and interactions with other conventional drugs and supplements. The challenge remains to improve patients’ knowledge and awareness of herbal medicines and dietary supplements and to indicate the possible harmful consequences of the self-prescribed use. It is necessary to change the attitude of patients and medical staff to these products in order to prevent side effects and increase safety. The health care staff needs to know about the use of any herbal medicines, pharmacotherapy anamnesis on hospitalization should include this information, and the clinical pharmacist should assess the overall therapy and make the right decisions. Overall, further research into hazards and risks of herbal medicines and other dietary supplements use in hospitals should be a priority.

References


Is there a place for rosuvastatin in the Lp(a) management?

Ana Vavlukis¹,²*, Marija Vavlukis³, Aleksandar Dimovski¹, Gordana Petrushevska³, Aleksandar Eftimov³, Sashka Domazetovska³, Almasa Demirovikj⁴, Kristina Mladenovska¹

¹Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, N. Macedonia
²Alkaloid AD Skopje, blvd. Aleksandar Makedonski 12, 1000 Skopje, N. Macedonia
³Faculty of Medicine, University Ss. Cyril and Methodius, 50 Divizija 6, 1000 Skopje, N. Macedonia
⁴PZU Ena-Medikal, Ferid Bajrami bb, 1000 Skopje, N. Macedonia

Introduction

Lipoprotein(a) [Lp(a)], an LDL-like particle, is an independent risk factor for cardiovascular disease (CVD). Recent studies indicate that people born with elevated Lp(a) may have a two-fold to four-fold increased risk of heart attacks and other serious CV events compared to people with low Lp(a) levels (Waldeyer et al., 2017). Clinical data points out that most people have Lp(a) levels in the range of 5 to 29 mg/dL, while the risk of CVD starts to rise at 30 mg/dL and more steeply at levels of 50 mg/dL.

While multiple studies have shown that Lp(a) is associated with elevated CVD risk, reducing Lp(a) has not yet proven to reduce CVD risk. Currently, there is no registered Lp(a)-targeted medicine. Treatment primarily includes niacin 1–3 g/day, while in extreme cases, LDL-apheresis. Literature data regarding the most widely used LDL-lowering drugs, HMG-CoA inhibitors/statins is controversial, showing (no) Lp(a) reduction, and even a slight increase (Tsimikas et al., 2019; Vavlukis et al., 2016). Among white JUPITER participants, Lp(a) was a significant determinant of residual risk, and relative risk reduction with rosuvastatin was similar among participants with high/low Lp(a) (Khera et al., 2014). The AIM-HIGH trial demonstrated that adding niacin to statin therapy reduces Lp(a) by 21%, but had no effect on CV events (Boden et al., 2011), while in the FATS study, Lp(a) was not associated with progression of atherosclerosis after statin therapy (Zhao et al., 2016). The aim of this study was to determine whether rosuvastatin has a potential role in regulation of increased Lp(a).

Materials and Methods

Adult outpatients without documented atherosclerotic CVD from the UKIM-University Clinic of Cardiology in Skopje were subjected to analysis (n=53, mean age 52.4±10.9 yrs; 34 (64.2%) females and 19 (35.8%) males). Of the 53 analyzed patients, 66% had hypertension, 22.6% diabetes, 50.9% pre-diabetes, 18.9% were smokers, 23.3% had positive family history for CVD and 63.2% had hyperlipidemia, without statistically significant gender differences. Mean EUROSCORE was 3.2±3.4% (intermediate risk), with males being in the high-risk category (5.0±3.8% vs. 2.3±2.7%, p=0.004); 11.8% females and 42.1% males were with high/or very high risk (p=0.027).

Of all patients, 81.1% had total cholesterol (TC) above 5 mmol/L at the study entry (no gender
differences), while pre-treatment lipid profile was as follows: TC 5.9±1.3 mmol/L; LDL-C 3.6±1.3 mmol/L; HDL-C 1.4±0.4 mmol/L; TG 2.0±1.1 mmol/L; ApoA1 1.6±0.3 g/L; ApoB 1.4±0.4 g/L and Lp(a) 48.1±73.0 mg/dL (range 7-342 mg/dL). 24.5% of the patients had Lp(a) levels above 50 mg/dL. The patients were treated medium term with rosuvastatin 20 mg/day (up to 20 weeks), when post-treatment lipid profile, HbA1c and fasting blood glucose (FBG) were determined.

Data was collected from patients’ records, physical examination and blood sampling. All parameters were measured by validated assays. Traditional risk-factors, calculated European System for Cardiac Operative Risk Evaluation Score (EUROSCORE), lipids and glycemic profile were analyzed as variables. Adequate indicators were statistically analyzed by Chi² test, pared sample t-test, Wilcoxon Signed Ranks test and correlations. Significance was determined at a level of <0.05.

Results and Discussion

Statistically significant decrease of TC, LDL-C, ApoB and TG was observed (mean difference as follows: -1.66±1.20 mmol/L, p=0.000; -1.23±2.48 mmol/L, p=0.001; -.041±.30 g/L, p=0.000; -.31±.76 mmol/L, p=0.004 respectively) after 20 weeks of treatment. However, statistically insignificant increase of mean Lp(a) level was observed (mean difference 5.07±44.73 mg/dL, p=ns) using pared sample statistics. Wilcoxon Signed Ranks test found 25 negative ranks (decrease after treatment), 23 positive ranks and 5 ties (p=ns). Even divided in two groups: normal Lp(a) (mean 18.95±12.85 mg/dL) and increased Lp(a) (defined as Lp(a) > 50 mg/dL i.e. 137.94±104.86 mg/dL), there was no statistically significant difference in both groups (28.93±53.69 mg/dL and 139.47±97.45 mg/dL, respectively, p=ns).

Furthermore, positive correlations for Lp(a) and: female gender (r=.514; s<0.000); LDL-C (r=.320; p=0.019); ApoB (r=.275; p=0.038) and CRP (r=.275; p=0.047), before statin treatment, were observed. However, analysis of the correlations after treatment demonstrated preserved correlation for Lp(a) only with female gender (-.374; p=0.006), explained by the treatment effect on TC, LDL-C and ApoB, but not on Lp(a).

After 12-20 weeks of treatment, small, however, statistically significant increase of HbA1c was observed (6.07±0.92% and 6.30±0.90% respectively, p=0.005), with no statistically significant difference in FBG (6.26±3.04 mmol/L and 5.69±2.09 mmol/L, respectively, p=0.059). It must be emphasized that this data was generated from the total study group, where 22.6% of the patients had diabetes.

Conclusion

The current study demonstrated no correlation between lipid-impact of rosuvastatin and its Lp(a) effect. In addition, there was no consistency of medium term rosuvastatin treatment on Lp(a) in “statin naïve” patients.

References

Perception of risk of adverse drug reactions with non-opioid analgesics by medical students

Zorica Stanojević-Ristić1*, Dragana Valjarević2, Aleksandar Ćorac1, Mirjana Dejanović1, Nenad Milošević1

1Faculty of Medicine, University of Priština-Kosovska Mitrovica, Anri Dinana bb, 38220 Kosovska Mitrovica, Serbia
2Faculty of Natural Sciences and Mathematics, University of Priština-Kosovska Mitrovica, Lole Ribara 29, 38220 Kosovska Mitrovica, Serbia

Introduction

Education about adverse drug reactions (ADRs) for public, healthcare professionals and medical students is the most important for reducing of drug-induced patient harm.

Some studies have already investigated patient’s knowledge about prescribed medicines (Cullen et al., 2006). Bongard et al. (2002) have shown major differences in the perception of risk of ADRs between health and non-health professionals. In this particular study, non-health professionals and pharmacists did not rank high non-steroidal anti-inflammatory drugs (NSAIDs) for perceived risk of ADRs compared to general practitioners and pharmacovigilance professional. Montastruc et al. (2003) found differences in the perception of risks of gastrointestinal ADRs with NSAIDs, including coxibs among physicians according to their medical specialization. Rheumatologists systematically considered NSAIDs as less harmful than general practitioners and gastroenterologists. This result may indicate that these physicians are involved more frequently in NSAID-induced ADRs than rheumatologists.

Differences in ADR risk perception has been illustrated in a study of French medical students (Durrieu et al., 2007). The study assessed the effect of education on students’ perception of ADR risks factors. Before taking a pharmacology course the students ranked NSAIDs in eight position, aspirin in twelfth position. After the course the order of risk perceptions changed to NSAIDs to fifth position and aspirin to fourth position. These results indicate significance of education on improving medication prescribing.

The aim of the study was to evaluate perceived risk of ADRs with non-opioid analgesics in young medical students and to investigate the impact of medical education on their perception of risk.

Materials and methods

A cross-sectional, questionnaire-based study was conducted among medical students of first and sixth year of study on Faculty of Medicine, University of Priština – Kosovska Mitrovica. The approval from Ethics Committee of the institution was obtained before the start of the study.

The study was conducted for the period of 2 months from April through May, 2019.

The total number of respondents was 205, of whom 45 were excluded because of incomplete data. The paper-based questionnaire was designed so that
the respondents could select more than one answer, or enter the appropriate data.

The questionnaire was divided into 3 sections. The first section included demographic information about students. The second section investigated the respondents’ perception of risk of ADRs next classes of non-opioid analgesics: paracetamol, aspirin, indomethacin for indolic derivatives NSAIDs, piroxicam for oxicam derivatives NSAIDs, diclofenac for arylcarboxylic derivatives NSAIDs and rofecocib for coxibs. The third section investigated the risk of ulcerogenic activity, gastrointestinal bleeding, liver and kidney damage, bronchospasm and thromboembolism associated with the use of each class of non-opioid analgesics.

A visual-analogue scale (VAS) was used to define a score for the perceived risk of ADRs. The scores were compared using Mann-Whitney U test. Differences were considered to be significant if the p value was less than 0.05.

Results and discussion

A total of 78 medical students of the first (MS1) and 81 medical students of the sixth (MS6) year of study completed the questionnaire giving the response rate of 78%. 63% of respondents were female and 37% were male. There was a significant age difference between the groups (19.6 vs. 23.6, p<0.001).

Both group of students ranked indolic derivatives of NSAIDs and coxibs as the most dangerous classes of non-opioid analgesics with high potentials for ADRs. Also, they considered that paracetamol was the least dangerous non-opioid analgesic. When NSAIDs and coxibs were considered together, the global median score of perceived risk of ADRs was 4.5 vs. 4.7 (MS1 vs. MS6), which was a statistically very significant difference (p<0.001). Clearly, at the end of their study, medical students become more cautious in the perception of risk of ADRs. Earlier studies have shown that NSAIDs were the pharmacological class most frequently involved in hospital admissions due to an adverse effect of a prescribed drug (Pouyanne et al., 2000).

For NSAIDs, MS6 gave significantly higher median scores of perceived “digestive risk” than MS1 (6.1 vs. 5.1, p=0.020). But unexpected, the perceived “thromboembolism risk” associated with the use of NSAIDs (including coxibs) was higher in MS1 than MS6 (5.8 vs. 4.8, p=0.007).

Conclusion

These results indicate that medical education gives young medical students a better understanding of the risks of potential ADRs. However, their perception of risk indicates the need for further education. Besides the influence of medical educations, other important factors, like information in the various media, have to be considered to explain the difference in the perception of risks related to drugs.

References


The Pharmacy simulation game- a unique global tool in pharmacy education

Tanja Fens¹,²*, Claudia Dantuma¹, Katja Taxis¹

¹Groningen Research Institute of Pharmacy, University of Groningen, Groningen, The Netherlands
²Department of Health Sciences, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

The need of effective educational concept for pharmaceutical practice

Pharmacists are recognized as key health professionals contributing to the health of citizens by providing professional advice on the safe, effective and rational use of medicines. Often, pharmacists are the first and the last contact patients have with the health system. To fulfill this role, pharmacy students need high quality education. In recent years, concept like simulation and serious gaming are increasingly used in the education of healthcare professionals (Cain and Piascik, 2015). Gradually, such concept is introduced in pharmacy education.

The Pharmacy Game in Groningen - GIMMICS®

GIMMICS® is an educational game, based on gaming theory and simulation, in which teams of students run a pharmacy for a period of a few weeks (Werf et al., 2004).

In Groningen, we teach our pharmacy students a number of competencies based on the Canadian Medical Education Directives for Specialists (CanMEDs). Since 2006, this competency framework is used in the educational programs of all Dutch medical specialists. The seven roles of the Dutch community pharmacist based on the CanMEDs model are: pharmaceutical expert, communicator, collaborator, manager, health advocate, scholar and professional (Westein et al., 2019).

The game is played in a simulated environment where students are managing their own pharmacy. Each pharmacy includes a counter, dispensing facilities and pharmacy information systems. Students have to counsel patients, process prescriptions, and communicate with other health care professionals. Patients are played by actors, voluntary staff members or externals. Furthermore, students have to perform tasks such as negotiations with insurance or wholesale companies, management of legal issues with drugs of dependence, drug-use-evaluations and submission of adverse drug reactions reports (Werf et al., 2004).

The Pharmacy Game internationalization

The game was developed in 2000, at the University of Groningen in the Netherlands. Until now, it has been adopted by six other Universities: University of Utrecht, Netherlands (2004), Vrije Universiteit Brussel, Belgium (2007), University of Nottingham, UK (2015), Griffith University, Australia (2016), University of Bath, UK (2018), and Vilnius University, Lithuania (2019) (More info is available at: www.gimmics.nl).

This is unique and possibly the only example of a pharmacy game which is taught in so many different universities. This internationalization
allows to share experiences between universities and working together towards better training of future pharmacists or other health workers.

**Evidence for using a simulation game in pharmacy education**

Educational gaming is a competitive activity engaging a set of rules which need to be followed. It allows active learning experience in a fun way advocating active participation and problem solving. Participants are able to train skills of independent decision making, learn from experience and peer communication, and develop a goal-oriented attitude (Akl et al., 2013). Evidence has also shown that simulation games enhance student’s communication skills. Furthermore, simulation in education allows real-life experience. Moreover, the students gain memorable learning, practical skills, and use experience to handle new situations and improve their performance. At the same time, it represents a safe learning environment within a controlled setting (Hasan et al., 2017; Pasquale, 2015).

Yet, in practice the use of games in medical practice remains dependent on evidence about the quality of its effectiveness and requires validation before implementation (Gorbanev et al., 2018).

The pharmacy simulation game GIMMICS® is practiced with success for 20 years now, focusing on the setting of community pharmacy. We have shown that it can be adapted to different health care contexts across the globe. It also allows to teach a wide range of different competences ranging from leadership, interprofessional collaboration to clinical competences. The concept could be also used to train other healthcare workers requiring practical experience e.g. family medicine (Van Rossem et al., 2019). Student evaluations indicate a preference for gaming teaching/learning methods compared to traditional teaching methods (Cain and Piascik, 2015; Eukel et al., 2017).

In conclusion, the Pharmacy simulation game represents a unique global tool for pharmacy education.

**References**


Devitalizing agents in endodontics

Ivona Kovacevska¹*, Mihajlo Petrovski¹, Olivera Terzieva-Petrovska¹, Ivo Kovacevski²

¹Faculty of Medical Sciences, Goce Delcev University, Krste Misirkov 10-a, 2000 Stip, Republic of N. Macedonia
²Alkaloid AD, 1000 Skopje, Republic of N. Macedonia

Introduction

Different types of pulpal inflammation mostly are presented as a sequel of dental caries. Most of the general dental practitioners find management of the inflamed pulp challenging in their routine dental practice. First step in the mortal endodontic methods of treatment is positioning of devitalizing medicament. The devitalizing agents can compose of formaldehyde, cresol, paraformaldehyde or some arsenic compounds. These agents can be harmful to the patients mostly because of their highly toxic, allergic, carcinogenic and mutagenic/genotoxic properties (Lewis, 2010).

Toxic devitalizing agents such as arsenic trioxide and paraformaldehyde were commonly used in the past to devitalize inflamed pulps when effective anesthesia could not be obtained (Ozmeric, 2002). Among these two substances an important role played paraformaldehyde pastes. Paraformaldehyde agents can play role as disinfectants and can devitalize inflamed pulps when local anesthesia is ineffective. Despite the clinical benefits, paraformaldehyde can penetrate through dentine and is gradually released as formaldehyde. Formaldehyde released through dentine has a destructive effect on periodontal and bone tissues (Mehmet et al., 2018).

In contemporary dentistry, formocresol is mostly used as an intracanal medicament because of its antibacterial and tissue fixative properties (Kunisada, 2002). Liquid formaldehyde compounds, such as formocresol may be expressed through patent apical, lateral and accessory canals and cause soft tissue and bony injury within the periodontium (Yakata et al., 1985).

Since the advantages of effective methods of local anesthesia, the use of paraformaldehyde or other toxic medicaments for pulp devitalization is minimized. However, contrary to the trends within modern endodontics, these medicaments are still using in the everyday dental practice and are sporadically associated with severe tissue breakdown in adults (Bataineh et al., 1997).

It must be noted that there is no latest reported literature available, concerning the behavior and attitude of general dental practitioners in relation to devitalizing agents.

Thus, the main aim of this study was to analyze knowledge, attitude and practice of general dental practitioners regarding the use of devitalizing agents in their respective practice.

Material and methods

Total number of 48 general dentists from the eastern part form our country were randomly selected. Adequate questionnaire was designed to cover general information of the participating dentist and concerning different aspects of devitalizing agents. General information was in place to record location/address, experience - years of practice and whether the practitioner is a part of teaching faculty in a dental school.

After the questions about the general information, questions regarding devitalizing agents were handed over to the professionals comprising 12 questions. The questions inquired as use of
devitalizing agents, type of agent used, purpose of use, frequency of use, usage in deciduous or permanent dentition, post-operative problems experienced (if any), frequency of the complications experienced, duration of use of devitalizing agent within the tooth, clinically observed changes in the tooth, reasons for usage and awareness regarding side effects/complications after use of devitalizing agents.

To all participants of this study were given adequate prior instructions necessary to fill the questionnaire. The questions were major in multiple choice questions, and the respondents were given the freedom to choose one or more suitable answers that meet their knowledge and attitude.

The collected data was statistic analyzed using SPSS (Statistical Package for Social Sciences) version 17.0. Descriptive statistics was drawn with respective percentages to have a comparative overview.

Results and discussion

More than half from the dentist (54.1%) were using devitalization agents. Of the respondents using devitalizing agents, 46.15% (or 25% from all respondents) were using agents containing arsenic, and 53.85% (or 29.17% from all respondents) were using agents containing aldehyde. 46% of the females and 46% of the males were using devitalizing agents, and the data showed that gender did not affect the usage of devitalizing agents (P>0.05). Also, it must be noted that there was a statistically significant difference in the usage of agents as related to the years of professional experience (P<0.05).

Nearly seventy percentage of general practitioners (precisely 68.75%) did not observe any post-operative complication following the use of devitalization agents. Post-operative pain was noted in 14.58% as the most frequently observed complication. Others noted complications were swelling in 8.33% and gingival necrosis 4.17%. 31.25% (15) of the respondents were not aware about the complications of devitalizing agents.

The use of aldehyde containing agents was more in the present study than other studies where they have used the recently available devitalizing agents like arsenic based compounds. The reasons reported by the practitioners for use of devitalizing agents is due to the fact that these agents are perceived to be quick and painless in action, eliminating the necessity of administration of local anesthesia thus saving time and maximizing patient cooperation (Unal et al., 2012).

Majority of dental practitioners did not observe post-operative complications, but few did. However, the existence of literature and the intensity of harm the patients experienced to date cannot be ignored. In addition, few respondents in the present study also observed post-operative complications in variety (Chen & Sung, 2014).

Conclusion

According to the results, it can be concluded that general dental practitioners do use pulp devitalizing agents in spite of possessing knowledge related to the complications.

References

Educational program strategy in rising the awareness for adverse drug reactions reporting and improved pharmacovigilance practice in the Republic of North Macedonia

Iskra P. Sadikarijo1*, Marijana Danevska1, Vera Georgieva1, Zorica Naumovska2

1Agency for drugs and medical devices, Blvd. SS. Cyril and Methodius 54, 1000 Skopje, N. Macedonia
2Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, N. Macedonia

Introduction

Pharmacovigilance (PV) is defined with the World Health Organization (WHO) definition as “the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other medicine-related problems”. Having undergone rapid growth over the past two decades, today, PV includes many other disciplines in the research and development of drugs. This growth has come resulted in heightened awareness and interest in the medical community about the roles that PV plays (Beninger, 2018). No country has a perfect pharmacovigilance system and we are facing underreporting globally. Well established PV system in the national level is inevitable for better patient care, improved public health, increased drugs safety and evaluation of risk/benefit ratio. Additionally, it is very important to be a part of global pharmacovigilance system in order to be up-to-date with all important issues concerning drugs utilized in everyday practice by the HCP and patients (Martin et al., 2018). Since 2015 National PV center in the Republic of North Macedonia is undertaken by the National competent authority - MALMED. This center works in accordance with the National regulative for pharmacovigilance, which is not fully harmonized with EU legislative Regulative (EU) 1235/2010, which was adopted in 2012, and since then it is continuously improved. Since 2002 Macedonia is a full member in the Uppsala monitoring center. Although the National center for pharmacovigilance exists for almost twenty years, the awareness for reporting adverse drug reactions (ADR) by the healthcare professionals and patients is still low (GVP, EMA). Since 2017 MALMED has established easy access to ADR reporting form for health care professionals (HCPs) and patients. Also state of art software system was grounded which enables easy and safe transfer of all reports in E2B R3 format according to EU legislative among all stakeholders, Agency, Marketing authorization holders (MAHs), Uppsala monitoring system (UMC) and Ministry of health.

This study aimed to evaluate the efficacy of implemented measures for improvement of knowledge and awareness of adverse drug reactions (ADRs) reporting among health care professionals (HCPs) including hospital pharmacists.

* iskra.sadikarijo@malmed.gov.mk
Materials and Methods

Training program as educational workshops for pharmacovigilance was organized and conducted by MALMED among 500 HCPs in twelve different cities. A community pharmacists were included from different regions from RN Macedonia. Subsequently non – interventional, questionnaire based study was enrolled in order to evaluate the improvement of knowledge and attitude toward ADR reporting and pharmacovigilance system. The official data were obtained from the Macedonian agency of drugs and medical devices (MALMED).

Results and Discussion

High percentage of awareness and knowledge for importance of ADR reporting was confirmed (over 95% of the participants), even before the implementation of educational program. Good theoretical understanding for pharmacovigilance was observed among HCPs and a positive attitude towards ADR reporting was presented by all participants. It was considered that ADRs reporting is contributing to utilization of safer drugs in clinical practice which is the major benefit from implementation of good PV practice. ADR reporting was associated with improvement of the quality of patient care and HCPs considered that the new information for the drugs obtained from good PV practice will contribute to rational use of drugs. All this will add to increased trust in the health service and help in improving the relationship between patients and health care professionals (HCPs).

Lack of sufficient information for the importance of ADR reporting, lack of knowledge whom to report or inappropriate fear of medical and legal issues were noticed as leading factors for underreporting. Additionally, the lack of time was addressed as the main limitation factor for implementation of appropriate PV practice, as well as by the uncertainty for association between occurred adverse drug event (ADE) and utilization of certain drug. The hospital pharmacists were nominated as main stakeholders in the improvement process for ADR reporting and have undertaken the responsibility for reporting toward Macedonian regulatory MALMED.

Conducted educational workshops with HCPs, improvement of theoretical knowledge alongside with practical skills for process of ADR reporting, development of website application by MALMED for easy access to reporting form for HCPs and patients, dedicated participation of all stakeholders resulted in huge progress in pharmacovigilance system and practice in the RN Macedonia. The number of reported ADRs in period of one year increased ten folds, 48 reports in December 2017 vs 505 in December 2018. In 2019 the number of submitted ADRs was over 300. This well established system is a key factor in improvement of PV practice and easy access for all HCPs and patients.

Conclusion

Implementation of appropriate strategy consisting of PV educational program with workshops alongside with contemporary software infrastructure was confirmed as a successful approach for improvement of ADR reporting in everyday clinical practices. Implemented educational and training program among HCPs with pivotal role of hospital pharmacist encouraged them for active participation in ADR reporting on the national level.

References

Module VIII Addendum I – Requirements and recommendations for the submission of information on non-interventional post-authorisation safety studies (Rev 2).

Orally disintegrating tablets (ODTs): a new approach to solid dosage forms

Tansel Comoglu

Department of Pharmaceutical Technology, Faculty of Pharmacy, Ankara University, 06100 Tandogan, Ankara, Turkey

Introduction

Orally disintegrating tablets (ODTs) are solid dosage forms that disintegrate usually less than a minute in the mouth into a paste that can be easily swallowed. ODTs have improved over the past years, in an attempt to produce a safe and efficient substitute to the conventional oral dosage forms. They are new types of dosage forms which mediate the advantages of both solid and liquid types of drug formulations such as ease of use and being stable. In this paper, advantages, ideal properties and desired characteristics of ODTs, formulation processes and future research trends in ODT technology will be told.

Advantages of ODTs

ODTs are especially convenient for patients, who have difficulties in swallowing conventional solid dosage form. ODTs include the following:

- Pediatric and geriatric populations who have complication in swallowing of tablets and capsules. ODTs are preferred especially by children, aged and bedridden people as well as the patients who wish to take their medicine at any time comfortably.
- ODTs are suitable dosage forms for during the journey, patients with permanent nausea.
- Antipsychotic drug molecules can be more easily applied to schizophrenic patients by ODTs than traditional pharmaceutical forms (Comoglu and Ozyilmaz, 2019; Velmurugan and Sundar, 2010).
- The risk of chocking or suffocation during oral administration of conventional formulations due to physical obstruction is avoided, thus providing improved safety (Indurwade and Biyani, 2000).
- Good mouth feel property of ODT helps to change the perception of medication (Allen et al., 1997).

Ideal properties of ODTs

An ideal ODT should maintain the following properties.

- Should be ionizable in oral cavity.
- Be dispersible and diffusible in mouth.
- The active material should be less than 50 mg in each tablet.
- Half-life of the active material should be short and suitable for frequently dosing.
- Should not have bad taste and smell.
- Should be dispersible in oral cavity without any need of water.
- Be robust to external conditions such as humidity and temperature.
- Conventional packaging processes can be applicable.
- Be able to manufacturing with using low cost equipment (Comoglu and Ozyilmaz, 2019).

* Tansel.Comoglu@pharmacy.ankara.edu.tr
Desired characteristics of ODTs

Because administration of ODTs is different from administration of conventional tablets, ODTs should maintain several unique properties (Sresta et al., 2017).

Fast Disintegration - ODTs should disintegrate in the mouth without additional water. The disintegrated tablet should become a soft paste or liquid suspension, which can provide good mouth feel and smooth swallowing.

Drug Properties - For the ideal ODT technology, the drug properties should not significantly affect the tablet property.

Taste of Active Ingredients - Taste masking is an essential requirement for ODTs for commercial success.

Tablet Strength and Porosity - Because ODTs are designed to have a quick dissolution time, excipients should have high wettability, and the tablet structure should also have a highly porous structure.

Moisture Sensitivity - ODTs should have low sensitivity to humidity. This problem can be especially challenging because many highly water-soluble excipients are used in formulation to enhance fast dissolving properties as well as to create good mouth feel. A good package design should be created to protect ODTs.

Formulation processes of ODTs

Manufacturing techniques of ODTs can be examined as patented and non-patented technologies.

Future research trends in ODTs

Although the ODT area has passed its infancy, as shown by a large number of commercial products on the market, there are still many aspects to improve in the ODT formulations. Despite advances in the ODT technologies, formulation of hydrophobic drugs is still a challenge, especially when the amount of drug is high. The future of ODTs also lies in the development of effective taste-masking properties. In general, the ODT formulations require large amounts of excipients, and having large doses of drug will only make the final formulation too big to handle. An ODT formulation that would require fewer excipients than the drug itself would be a breakthrough. While the problems to be solved are not easy, the history suggests that it is just a matter of time before they are solved (Fu et al., 2004).

Conclusion

ODTs have improved patient compliance, convenience, bioavailability and rapid onset of action. In future, ODTs may be the most acceptable and prescribed dosage form due to its rapid action. Their characteristic advantages such as administration without water, anywhere, anytime lead to their increased patient compliance in today’s scenario of life. Considering many benefits of ODTs, a number of formulations are prepared in ODT form by most of the pharmaceutical companies. Because of increased patient demand, popularity of these dosage forms will surely expand in future (Sresta et al., 2017).

References


Pharmaceutical uses of graphene

Despina Spasevska Kovacki

Alkaloid AD, Skopje, Blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia

Introduction

Graphene is a material composed of pure carbon, similar to graphite but with characteristics that make it extraordinarily light and strong. A sheet of one square meter of graphene weighs 0.77 mg. Its strength is 200 times greater than that of steel and its density is similar to that of carbon fiber. All these make it resist high bending forces without breaking. It is one of the most conductive materials for electricity and heat, which makes it the perfect material for many industries.

Graphene oxide (GO), the oxidized form of graphene, holds great potential as a component of biomedical devices, deriving utility from its ability to support a broad range of chemical functionalities and its exceptional mechanical, electronic and thermal properties.

Some of the uses of graphene in pharmacy are listed in the following text.

Wang et al. (2019) have shown that graphene-based nanomaterials (GNMs) are successfully used for the removal of prominent antibiotics, which absorb onto GNMs mainly through π-π interaction, electrostatic interaction, hydrophobic interaction and hydrogen bonding.

Graphene is a magnificent bactericidal material as it avoids the generation of microorganisms, such as bacteria, viruses, and fungi, by damaging their cell membranes between its outer layers. When compared to different derivatives of Graphene, Graphene Oxide and reduced Graphene Oxide shows the best antibacterial effects. GO can also be used as a compound with silver nanoparticles to increase antibacterial properties even further (Song et al., 2016).

Functionalized graphene can be used to carry chemotherapy drugs to tumors for cancer patients. Graphene based carriers target cancer cells better and reduced and decreased toxicity of the effected healthy cells.

Patel et al. (2016) have shown that hydrophobic cancer drug delivery is facilitated by hydrophobic interactions between the drug and graphene oxide molecules, leading to water dispersible graphene loaded with cancer drugs with otherwise limited water solubility. It is also an excellent photothermal agent for photothermal therapy. Its physiochemical properties can be harnessed to facilitate photothermal and photodynamic therapy to treat superficial or deep tissue cancers.

In vitro investigations and in vivo proof of principle small animal studies provide evidence that graphene-based formulations could serve as versatile delivery agents. The results suggest that morphology of graphene as well as the functional groups employed to improve its water dispersibility may play an important role toward the development of an optimum formulations for efficacious anticancer theropies.

Drug delivery is not limited to cancer treatment. Anti-inflammatory drugs have also been carried by graphene and chitosan combinations and yielded promising results (Unnithan et al., 2017).

* dkovacki@alkaloid.com.mk
Gene delivery is a method used to cure some genetic diseases by bringing foreign DNA into cells. Graphene oxide (GO) modified by polyethyleneimine can be used for these purposes is expected to show low cytotoxicity, as it did in the drug delivery case (Bao et al., 2011).

Holt et al. (2016) have shown that GO or a GO-containing composite can be used to coat traditional osteogenic materials/scaffolds. GO can be noncovalently or covalently loaded for controlled release of osteogenic molecules. GO can be incorporated with other materials to form composites with enhanced properties for osteogenesis.

Graphene oxide/collagen (GO-COL) aerogel exhibit relatively good biocompatibility and osteogenic ability in vitro and in vivo, which make it a promising bioscaffold for bone tissue engineering and regenerative medicine (Liu et al., 2019).

A group of researchers (Kidambi et al., 2017) showed that graphene (in a form of graphene membrane) can be used to filter the blood from wastes, drugs and chemicals as well. Graphene’s superiority in this case is that it is 20 times thinner than traditional membranes which leads to significant decrease in the time spent in the dialysis for the patients.

An injectable hydrogel scaffold made of polyethylene glycol-melamine-hyaluronic acid-GO (PEG-MEL/HA-SH/GO) was found to have excellent conductivity and anti-fatigue properties for cardiac tissue engineering. When injected into the infarcted area of the myocardium, adipose-tissue-derived stromal cells encapsulated within these PEG-MEL/HA-SH/GO hydrogels improved the functional performance of the heart (Lakshmanan and Maulik, 2018).

Conclusion

The application of graphene is virtually unlimited and promises to revolutionize many fields: from electronics and computing to construction and health.

Graphene is called the “wonder material” for a reason. There are tens of researches about it which are not published yet, but may change the world tomorrow.

References


Compounding of $^{99m}$Tc-labeled antimicrobial peptide for molecular imaging of bacterial infections

Sonja Kuzmanovska*, Venjamin Majstorov

Institute of Pathophysiology and Nuclear Medicine, Medical Faculty, Ss Cyril and Methodius University, Mother Teresa 17, 1000 Skopje, Republic of North Macedonia

Introduction

In clinical practice it is often very challenging to discriminate bacterial infection in bone, soft tissue and orthopedic devices from sterile inflammation, due to the common unspecific symptoms. The most useful diagnostic procedures for detecting infection in such conditions are histopathology and imaging studies.

Nuclear Medicine imaging techniques have been widely used to detect functional, metabolic, as well as biochemical changes at molecular level. In vitro radiolabeled autologous leukocytes have been considered “gold standard” for visualization of suspecting infections (Palestro et al., 2009), especially in orthopedic surgery. Besides $^{99m}$Tc and $^{111}$In – labeled leukocytes, used as single photon emitting (SPECT) radiopharmaceuticals (RF), $^{18}$F-FDG and recently $^{64}$Cu as positron emitting tomography (PET) labels are introduced to improve the quality of leukocyte imaging studies (Rini and Palestro, 2006). Nevertheless, they have all been proved to be non-specific in discrimination between infection and sterile inflammation (Bunyaviroch et al., 2006).

Radiolabeled antimicrobial peptides (AMPs) are new generation of radiopharmaceuticals, developed to overcome the lack of specificity issue. One of these peptides is derivate (29-41 fraction) of ubiquicidine (UBI), a natural AMP found in all mammal tissues, which specifically binds to bacterial membrane, enters the cell and remains within the cytoplasm. Our aim was to present the compounding of $^{99m}$Tc-UBI (29-41) and quality control of the radiopharmaceutical, as primary step of product introduction in Nuclear Medicine Department settings.

Materials and methods

The study was carried out at the Instituto Nacional de Investigaciones Nucleares (ININ), Mexico. The ligand UBI (29-41), used for the kit production and the compounding process, was purchased from Bachem Bioscience Inc. Radiolabeling with $^{99m}$Tc was performed by direct procedure, utilizing two protocols: freeze-dried kit formulation labeling (Ferro-Flores et al., 2005) and “in-situ” unit dose labeling (Ferro-Flores et al., 2003). $^{99m}$Tc as sterile, apyrogenic Na$^{99m}$TcO$_4$ solution was obtained from GETEC $^{99}$Mo/$^{99m}$Tc generator (ININ, Mexico).

$^{99m}$Tc UBI freeze-dried kit compounding

UBI freeze-dried kit is two vials commercial product of ININ (Mexico), containing 25μg UBI (29-41) and 12,5μg SnCl$_2$ in the first and 40 μL 0,1 M NaOH in the second vial. In brief, 370-740 MBq of Na$^{99m}$TcO$_4$ was added to the second vial and the alkalized label was afterwards transferred to the first
vial. After incubation of 15 minutes, intermediate pH was measured. The volume was then adjusted to 3mL with 0.9% saline and final pH measured.

\textit{99mTc UBI “in-situ” unit dose compounding}

For the “in-situ” compounding we assessed three different formulations with constant amount of reducing agent and variable amounts of ligand and alkalizing agent. UBI aqueous solution (1mg/200 µL), SnCl\textsubscript{2} solution (1mg/mL) and 0.1M NaOH had been prepared prior the compounding. Formulation 1 was compounded with 50 µL UBI, 25 µL SnCl\textsubscript{2} and 50 µL NaOH, followed by 100 µL of 370-740 MBq Na\textsuperscript{99mTc}O\textsubscript{4}. Formulation 2 consisted of 50 µL UBI, 25 µL SnCl\textsubscript{2}, 20 µL NaOH, and 100 µL of 370-740 MBq Na\textsuperscript{99mTc}O\textsubscript{4}. For formulation 3 we used 25 µL UBI, 25 µL SnCl\textsubscript{2}, 10 µL NaOH and 100 µL of 370-740 MBq Na\textsuperscript{99mTc}O\textsubscript{4}. After 5 min. incubation, volume was adjusted to 3 mL with 0.9% saline. Intermediate and final pH was measured. Before patient administration, the RF should be membrane filtrated trough 0.22 µm

Radiochemical purity was determined in both-kit and “in-situ” RFs by instant thin layer chromatography (ITLC) on silica gel in 0.9% saline as mobile phase. Additionally, reverse phase HPLC on a C-18 column with radioactivity detector was used, as described (Ferro-Flores et al., 2003).

\textbf{Results and discussion}

Successful \textsuperscript{99m}Tc-UBI compounding process and the stability of the RF are dependent of alkaline pH. During the \textsuperscript{99m}Tc UBI freeze-dried kit compounding, the formation of radioactive complex occurred at pH 9. After adjustment with saline, final pH was 7.5. In all three “in-situ” compounded formulations the pH value, measured after incubation, was between 9 and 10. After obtaining the final volume, the pH remained within the same range.

Average radiochemical purity (RCP) of \textsuperscript{99m}Tc UBI freeze-dried kit determined by ITLC was 97.3±1.1% (N=3) (Rf \textsuperscript{99m}Tc-UBI = 0.0; Rf \textsuperscript{99m}TcO\textsubscript{4} = 1.0). RCP determined by HPLC (retention time of 12.5±0.5 minutes for \textsuperscript{99m}Tc UBI) was 98.8±1.3% (N=3).

Quality control results obtained for the \textsuperscript{99m}Tc UBI “in-situ” formulations reveal high RCP in all 3 RFs and had not been affected by the increased quantity of the ligand and alkalizing agent. RCP by ITLC and HPLC was 97.9-98.6% and 99.5-99.8% respectively.

\textbf{Conclusion}

\textsuperscript{99m}Tc UBI compounding was successfully performed with freeze-dried kit and “in-situ” formulations, both with high PCP. In Nuclear Medicine Department settings without GMP premises for in-house kit production, compounding of the RF could be performed in Shielded Biosafety LAF cabinet using “in-situ” unit dose formulations.

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\textbf{References}


Design of skin-simulating nanoformulations for ceramide replacement in the skin: a preliminary study

Hümeyra Şahin Bektay*, Emine Kahraman, Sevgi Güngör

Department of Pharmaceutical Technology, Faculty of Pharmacy, Istanbul University, 34116 Istanbul, Turkey

Introduction

Ceramides are lipid components, which contribute for unique barrier property to outermost layer (stratum corneum) of the skin. They exist in intercellular lipid domain of stratum corneum, at approximately equimolar concentrations of free fatty acids, cholesterol, and ceramides (Coderch et al., 2003). Furthermore, ceramides play a crucial role in structuring and maintaining of barrier function of the skin. Dermatologists also revealed that total levels of ceramides decrease with some differences in ceramide pattern of stratum corneum for certain skin disorders such as psoriasis, atopic dermatitis with compromised skin barrier function, then trans-epidermal water loss (TEWL) and pH of the skin increase. Then, redness and itching are observed in the patients (Kahraman et al., 2019).

In the light of this information, it has considered that the formulations consisting of ceramide could improve impaired skin disorders. Additionally, it is well known that replacement of endogenous stratum corneum lipids accelerates skin barrier recovery (Coderch et al., 2003). Recently, Ishida et al. (2020) reported that synthetic pseudoceramide significantly decreased skin symptoms, reducing in TEWL and raising in water content, as a result clinically provided to transport ceramide profile from an atopic dermatitis to a healthy skin phenotype. Thus, a numerous of commercial lotions, creams, and moisturizers (Eucerin Smoothing Repair Dry Skin Lotion, Eucerin Eczema Relief Body Creme, CeraVe Moisturizing Lotion) consisting of ceramide I and III have been formulated in the market (Spada et al., 2018). However, high lipophilicity of ceramides may impede their penetration to stratum corneum and reach into lipid lamellae when applied conventional topical formulations (Deli et al., 2009). To overcome this issue, some researchers studied ceramide loaded nano-carriers (Deli et al., 2009; Tessema et al., 2018), although not enough.

In this study, we aimed to develop skin-simulating nanoformulations for ceramide replacement in the skin. In this context, ceramide loaded liposomes, ultra-deformable vesicular systems (transethosomes) and micelles were prepared and optimized in terms of particle size and polydispersity index (PDI, size distribution).

Materials and methods

Preparation of liposomes, transethosomes and transferomes

In preparation of the liposomes, Phospholipon 90G (Lipoid GmbH, Germany), cholesterol (Sigma-Aldrich, USA), and ceramide III (Evonik Ind. AG, Germany) were dissolved in a mixture of methanol and chloroform (1:1, v/v). Then, residual solvents were removed by a vacuum evaporator and a thin film layer generated in a round-bottom flask. The film layer was hydrated with ultrapure water. The formulation was sonicated for diverse times at 20
MHz of frequency. The same procedure was applied for the transehtosomes and transfersomes. Just only, Tween 80 was facilitated instead of cholesterol for the transehtosome and transfersome formulations. Also, a mixture of ethanol and ultrapure water (3:7, v/v) was used as dispersion medium in the transehtosome formulations.

Preparation of micelles

D-α-Tocopherol polyethylene glycol 1000 succinate (TPGS) (BASF, Germany) super refined oleic acid (Croda, UK), cholesterol and ceramide III were dissolved in methanol. A thin film layer was obtained in a round-bottom flask after evaporating of organic solvent. Afterwards, the film layer was hydrated with ultrapure water.

Particle size and PDI

The particle size and PDI of vesicular systems and micelles were measured using dynamic light scattering by NanoZS ZetaSizer (Malvern Instruments, UK) at 25.0±0.1 °C.

Results and discussion

The particle size and PDI value are importance keys for optimization of nano-carriers (Kahraman et al., 2016). Thus, these parameters were examined to optimize a feasible nanoformulation simulating the skin. In all of the liposome formulations, micro-scale size and high PDI value were determined. Assessing the transehtosomes formulations, it was demonstrated that Tween 80 improved size and PDI of nanocarriers in comparison with cholesterol. The formulation containing of ceramide III, Phospholipon 90G and Tween 80, which sonicated for 3 min, exhibited the smallest size (approximately 100 nm) and the narrowest size distribution (0.086). The particle size and PDIs of transfersomes, which were prepared at similar constituent ratios, were measured about 60 nm and approximately 0.4, respectively. In the transfersome formulations, this case may be explained absence of ethanol, reducing polarity of dispersion medium and increasing ceramide solubility.

When the micellar formulations were evaluated, it was revealed that formation of TPGS micelles was negatively affected in presence of oleic acid, cholesterol and ceramide in the dispersion medium, separately. Thus, ceramide loaded micelles could not be prepared and visible aggregates were viewed in the formulations.

Conclusion

As a result, transehtosomes consisted of Phospholipon 90G, Tween 80 and ceramide III, could be a feasible formulation for ceramide replacement in the skin, because of small size and narrow size distribution. However, more characterization studies and in vivo experiments are required to assess conformity of this formulation.

References


Introduction

Ibuprofen (IBU) is commonly used non-steroidal anti-inflammatory drug. It is a weak acid (pKa ~4.5) with low pH dependent aqueous solubility (46 μg/mL at pH 1.5 and >300 μg/mL at pH>7, at 25 °C). The most commonly used oral dose is 200–600 mg/6h. Drug solubility, which affects the dissolution and absorption from the formulation is a common problem in developing efficient formulation for oral IBU delivery (Ćirić et al., 2020; Irvine et al., 2018).

Chitosan (CH) and xanthan gum (XG) polyelectrolyte complexes (PECs) already demonstrated improved drug solubility, permeability, pH sensitivity and controlled drug release. Polycationic CH can interact electrostatically with negatively charged compounds (Sogias et al., 2012), such as XG, for the development of PEC-based drug carriers.

IBU encapsulation procedure could influence the drug-polymer interactions and the PECs formation. The aim of this study was to evaluate the influence of IBU encapsulation procedure on the formation and properties of CH/XG PECs as drug carriers.

Materials and methods

Three different procedures of IBU (BASF, Germany) encapsulation were performed. In the procedure A, IBU was mixed with formed PEC hydrogel consisting of medium molecular weight CH (Sigma Aldrich, USA) and XG (Jungbunzlauer, Switzerland) (4.6A). In the procedure B, IBU was dispersed in the XG solution before mixing with the CH solution and PEC formation (4.6B). In the procedure C, IBU was added into the aqueous medium after the complexing of CH and XG and allowed to diffuse into the PEC (4.6C). The concentration of both polymers in the aqueous solutions was 0.65% w/v, and their volume ratio 1:1. The pH of CH solutions was adjusted to 4.6 with acetic acid. Mixing was performed on laboratory propeller mixer RZR 2020 (Heidolph, Germany). IBU-to-polymers mass ratio was 1:1.

The evaluation of PECs formation and the strength of interactions between the polymers and IBU was done by pH (HI 9321, Hanna Instruments, USA), conductivity (CDM 230, Radiometer, Denmark) and rheological measurements (Rheolab MC 120, Paar Physica, Austria) by increasing the shear rate from 0 to 100 s⁻¹ and back to 0 s⁻¹ at 20±0.2 °C, in triplicate.

PEC hydrogels were dried under ambient conditions, grinded and sieved. Then, the yield (%Y), the IBU encapsulation efficiency (%EE) and the drug loading (%DL) were calculated. The total amount of CH, XG and IBU, the initial amount of IBU used for PEC preparation and the IBU/polymers
ratio were considered 100% for %Y, %EE and %DL, respectively. The calculation of %EE and %DL: 20 mg of each PEC was dissolved in 100 ml of methanol/phosphate buffer pH 7.2 (80:20 V/V) by sonication (Sonorex RK1024, Bandelin, Germany). IBU concentration was determined spectrophotometrically at 224 nm (Evolution 300, Thermo Scientific, USA).

Results and discussion

After the complexing of CH and XG and the encapsulation of IBU, the pH values of 4.33±0.05 for 4.6A, 4.49±0.05 for 4.6B and 4.11±0.03 for 4.6C were measured. The lowest pH of 4.6C can be explained by the diffusion of hydrogen ions into the hydrogel from the PEC preparation medium, due to IBU dissociation. The highest pH for 4.6B could be explained by the dispersion of IBU in XG solution at high drug concentration. That suppresses its dissociation, resulting in lower concentrations of hydrogen ions into the hydrogel. The ionization ability of IBU, responsible for its non-covalent interactions with CH and XG, may accelerate the dissolution of this crystalline drug by partial disruption of its crystal lattice, which could potentially influence the release kinetics of IBU from PEC-based carriers (Sogias et al., 2012).

The conductivity decreased during the formation of PECs, confirming the establishment of interactions between the polymers and IBU. The final conductivity of 4.6A was 920±1 μS/cm, of 4.6B was 615±3 μS/cm, and of 4.6C 833±67 μS/cm. The differences between the samples were expected since only free ions are responsible for the conductivity of samples (Čirić et al., 2020).

All PECs showed pseudoplastic flow behavior with thixotropy. Thixotropy was evaluated based on the hysteresis area (H) values. The highest value of 1019.10±297.01 Pa/s was detected for 4.6B, while the lowest, 68.20±47.39 Pa/s, was measured for 4.6A. The H of 4.6C was 784.06±143.63 Pa/s. Higher H values are correlated with stronger interactions between IBU and polymers (Čirić et al., 2020; Djekic et al., 2016). The strength of the interactions was also evaluated by measuring the apparent viscosity (maximal at 11.1 s⁻¹ – ηmax, and minimal at 100 s⁻¹ – ηmin) of PECs. The highest ηmax was measured for 4.6B (4.97±0.43 Pa·s), and the lowest for 4.6A (3.92±0.36 Pa·s). The ηmax for 4.6C was 4.30±0.23 Pa·s. The ηmin for all samples was 0.64±0.08 Pa·s. These results are in accordance both with thixotropy and the conductivity of the samples.

The highest %Y and %EE had 4.6B (54.14±3.14% and 59.05±3.14%, respectively), and the lowest 4.6C (18.70±4.23% and 0.89±0.05%, respectively). For 4.6A, %Y was 48.04±2.08%, and %EE 53.19±3.07%. %DL ~50% for 4.6A and 4.6B and ~2.5% for 4.6C indicated that the PEC 4.6B resulted in the best characteristics as a carrier of poorly soluble, highly dosed drug, such as IBU.

Conclusion

IBU encapsulation by dispersion in the XG solution before mixing with the CH solution (PEC formation) could be considered optimal to prepare PECs as promising drug carriers with strongest interpolymer interactions, the highest %Y, and %EE.

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References


The effect of Kanjiža peloid on skin hydration and skin barrier function

Gábor Katona1*, Sladana Vojvodić1, Marina Kalić1, Miroslav S. Sarač1, Attila Klimó2, Nataša Jovanović Lješković1

1Faculty of Pharmacy Novi Sad, University Business Academy in Novi Sad, Trg mladenaca 5, 21000 Novi Sad, Republic of Serbia
2Special Rehabilitation Hospital "Banja Kanjiža", Národni park bb, 24420, Kanjiža, Republic of Serbia

Introduction

Peloid is a mixture of mineral water, organic and inorganic substances, as a result of geological and biological processes by definition of the International Society of Medical Hydrology (Potpara et al., 2017). Due to unique characteristics, a combination of biological and chemical components, the peloid exhibited strong antibacterial and anti-inflammatory effects (Devdariani and Jinchararadze, 2018; Leshchinskiĭ et al., 1972). Also, there are evidence of peloid’s positive effects on dermatological conditions such as psoriasis (Costantino and Lampa, 2005), acne and seborrhea (Szabó and Töröcsik, 2016), as well as its benefits for skincare such as cleansing, degreasing, exfoliating, hydrating, tonifying (Carbajo et al., 2010; Rautureau, et al., 2017). Therapeutic benefits of peloid are especially recognized in the field of rheumatology and dermatology.

The rehabilitation hospital, "Banja Kanjiža," has used peloid for rehabilitation treatments, and it is currently in use. This observational study aimed to determine the potential use of the Kanjiža peloid for cosmetic purposes by testing the effects of this particular peloid on skin hydration and transepidermal water loss (TEWL). There are many published literature data about the other peloids worldwide utilized for the treatment of cosmetological and dermatological conditions; however, this is the first time the Kanjiža peloid was investigated for cosmetic purposes.

Materials and methods

The peloid and thermal water used in this study were obtained from “Banja Kanjiža.” The formulation of a peloid and thermal water-based cosmetic product (paste) was conducted at the Faculty of Pharmacy Novi Sad, Novi Sad, Republic of Serbia. The IRB – Ethics committee approved the research study. The research design is quantitative, quasi-experimental with pre-test, and post-test assessment and measurements.

Human subjects (n = 31) of both genders, age 18 - 60 with all skin types, were included in the study. Exclusion criteria were: females who are pregnant or breastfeeding, those who have current active skin diseases or skin disorder in the forearms, those who are allergic/sensitive to peloid or contemporary cosmetic products. Human subjects provided their consent to participate in the study by using a signed and witnessed informed consent form strictly voluntarily and their free will. Eligible subjects were instructed not to apply any cosmetic products on
their forearm skin at least 24 hours before the study commencement.

The pre-test included skin hydration and TEWL assessment. Before the assessments being performed, the study participants acclimatized to study laboratory conditions (40 ± 4% RH and a temperature of 20 ± 2 °C) for a minimum of 20 minutes. Multiprobe adapter MPAS® with Corneometer® CM825 probe and Tewameter® TM300 probe (Courage-Khazaka electronic GmbH, Germany) was used for non-invasive measurement of skin hydration and TEWL on the forearm epidermis.

The treatment conducted in this study was non-invasive: using a plastic spatula, a small amount of peloid was smeared on a 4 x 5cm surface area of the forearm skin of human subjects. The treatment ended after 30 minutes by washing off the peloid from the human subject’s forearm skin.

The post-test (after 20 minutes of acclimatization to studying laboratory conditions) included the same protocol as for the pre-test: skin hydration and TEWL assessments.

**Results and discussion**

Corneometer® CM825 provided us with results that show the peloid did not cause the skin to become dry (X̄ of pre-test 30.3; X̄ of post-test 29.0; t-test 0.1774). This phenomenon could be explained by the forming of a peloid shield (Carretero, 2001). Tewameter® TM300 data, on the other hand, exhibited that the peloid caused a slight increase in TEWL values: X̄ of pre-test 8.48; X̄ of post-test 16.95; X̄ increased 199.8%, but the skin barrier function has remained without redness, irritation, and sensitivity. The exfoliating effect of the peloid could be behind this occurrence. Peloid is rich with a variety of crystal structures such as silicon and other compounds (Rautureau, et al., 2017), which possess the ability to remove dead cells layer of stratum corneum and cause the increase of the values measured by Tewameter®. This result is in correlation with literature data regarding potential the peloid’s exfoliating effect (Rautureau, et al., 2017).

**Conclusion**

The results of this observational study exhibited that the Kanjiža peloid and thermal water are potentially suitable to be pharmaceutically and cosmetologically processed, related to the peloid's exfoliating effect and possible prevention of dehydration. The results of this preliminary observational study are contributing to further testing of this natural material, such as the effect on the amount of sebum to reveal potential anti-comedogenic (Carretero, 2001) and regenerative effects of cosmetic products made explicitly from the Kanjiža peloid.

**References**


Development and evaluation of bee venom topical formulation for efficient treatment of arthritis

Lejla Mutapcic1,2, Tamara Ivanoska1, Angela Mircevska1, Eleonora Trajanovska1, Ljubica Mihailova1, Dushko Shalabalija1, Nikola Bijeljanin3, Midhat Jasic2, Maja S. Crcarevska1, Marija Glavas-Dodov1*

1 Faculty of Pharmacy, UKIM-Skopje, Majka Tereza 47, 1000 Skopje, N. Macedonia
2 Faculty of Pharmacy, UNTZ, Univerzitetska 8 Tuzla, BiH
3 Bee cultivator Leshnica, 1000 Skopje, N. Macedonia

Introduction

Arthritis is a chronic, complex autoimmune disease that affects approximately 1% of the global population. Conventional therapeutic management involves usage of steroids, nonsteroidal anti-inflammatory, disease modifying antirheumatic and immunosuppressant drugs. Despite the increasing number of new drugs and treatment regimes, complete long-term disease remission is not achieved for many patients and thus new therapeutic options are required (Guo et al., 2018).

Bee venom (BV) therapy has been used since ancient times. According to animal experiments, BV exhibits antiarthritic, anti-inflammatory and analgesic effects attributable to the suppression of cyclo-oxygenase-2 and phospholipase A2 expression and a decrease in the levels of TNF-α, IL-1 and IL-6, nitric oxide and oxygen-reactive species. Bioactive BV compounds, such as peptides (melittin, adolapin and apamin), enzymes (phospholipase A2) and amines are also associated with these actions (Lee et al., 2014).

The topical delivery is an attractive method for local treatment of inflammatory conditions like musculoskeletal disorders. Topical delivery has many advantages over the conventional oral dosage forms, especially in avoidance of various adverse effects. Having in mind that the therapeutic efficacy of a topical formulation depends on both the nature of the vehicle and the physicochemical properties of the active agent (release rate, rate and extent of drug permeation, etc.) (Özcan et al., 2009), the aim of this study was to develop an effective, stable topical gel formulation containing BV as an active agent.

Materials and methods

BV was collected by electric stunning, without harming honey bees during July 2019 (Kozarac, BiH) and was stored at -20°C. A modified HPLC method (Rybak-Chmielewska and Szczêsna, 2004) was used for assay of BV using melittin (Sigma, USA) as an external standard (Agilent Technologies 1200 Series; Restek Ultra C18 column; gradient elution with 0.1% trifluoroacetic acid (TFA) in water (mobile phase A) and 0.1% TFA in acetonitrile-water 80:20 (mobile phase B), flow rate 2.5 mL/min, 20 μL injection volume, λ of 220 nm).

The gels were prepared by dissolving different concentrations of chitosan (CTS, low-molecular weight; Sigma–Aldrich, USA) in 1% of lactic acid...
solution with or without poloxamer 407 (PL; Pluronic F127, BASF Chemtrade GmbH, Germany) (1.75% CTS+0.5% PL-sample 1, 2% CTS+0.5% PL-sample 2, 1.75% CTS-sample 1O, 2% CTS-sample 2O, respectively), 5% of propylene glycol (Alkaloid, N. Macedonia), 0.2% of potassium sorbate (PS; Apac Chem. Corp., USA) and 0.3% of BV by agitation at 300 rpm with the aid of magnetic mechanical stirrer (Variomag, Germany).

Prepared gels were characterized for pH (Sartorius, Germany), viscosity (at 25 °C; DV2T, Spindle T bar T-A 91, Brookfield Eng. Lab., Inc., USA) and spreadability (0.5 g of gel covered with 68 g glass plate, during 5 min). PS content was determined UV spectrophotometrically (264 nm; Lambda 16, Perkin Elmer, USA) after dissolving of 1 g of gel in 10 mL of methanol. In vitro BV release from the gels (1.5g) was performed using membrane diffusion cells (MEMBRA-CELL dialysis tubing; Serva Feinbiochemica GmbH, Germany) (32±0.5 °C, 15 mL of distilled water as a dissolution medium, 300 rpm). At predetermined time intervals aliquots were taken and analyzed by HPLC.

**Results and discussion**

BV assay and quality were determined based on melittin content. Obtained BV sample contained 43.54% of melittin. Prepared gels were characterized with the pH of 4.5-5.2. Having in mind that the consistency is one of the most important features for analgesic and anti-inflammatory topical forms, the gel viscosity plays an important role in drug permeation control. Results from the viscosity measurements showed that by increasing the CTS concentration, the viscosity of the samples increased (5920 cP for sample 1O and 11620 cP for sample 2O, respectively). By incorporation of 0.5% PL, the viscosity of the gels also increased, probably due to PL properties for micellization and sorting the polymer chains into a denser network (6400 cP for sample 1 and 18120 cP for sample 2, respectively). All prepared samples showed pseudoplastic behavior. The therapeutic efficacy of gels also depends on their spreadability. The proper spreading helps in the uniform application of the gel to the skin and satisfies the ideal quality for topical application. Spreadability factor calculations demonstrated that sample 2O had the significantly higher spreadability in comparison to sample 1O (p<0.05). By incorporation of PL, both formulations (sample 1 and sample 2) showed similar spreadability as sample 2O. The content of potassium sorbate in formulated gels was 99.85±2.04%. The concentration of gelling agents (CTS or CTS/PL combinations) significantly influenced the in vitro release behavior of BV from the prepared formulations. Gel formulation prepared with 1.75% CTS and 0.5% PL (sample 1) showed highest release rate (~98%) during the period of 24 h, following the Peppas-Sahlin kinetic model.

**Conclusion**

According to the results obtained from this study it could be concluded that BV was successfully incorporated into the CTS/PL gels formulations. Prepared gels showed suitable pH value, viscosity and spreadability. Formulation prepared with 1.75% CTS and 0.5% PL could be a promising candidate for efficient topical delivery/treatment of arthritis. Further clinical studies should be conducted.

**References**


Potential irritants and allergens in shampoos-type preparations

Isidora Milanović¹*, Jasmina Bašić¹, Danijela Pecarski¹, Dragana Dragaš Milovanović¹

¹Belgrade Academy of Professional Studies, Department Medical College of Professional Health Studies, Cara Dušana 254, Zemun, Belgrade, Serbia

Introduction

The shampoos have the role to cleansing impurities from skin and hair surface at relatively low temperatures and for a short time. The shampoos need to have a beneficial effect on the scalp and hair, also prevent them from being damaged by external and internal factors. The shampoos use surface active agents (SAAs) with an HLB value of 13-15 (according to Griffin scale), as a primary, which provide good washing, foaming, and removal of impurities with friction. They reduce the surface tension between impurities and water; peptize, emulsify or solubilize and remove them with the foam, rinsing with water. The secondary surfactants in shampoos are poorly foamed, wash well and have a lower irritant potential (Rulebook of cosmetic products, 2019).

The most commonly used as a primary surfactants are sodium lauryl sulfate (SLS) and sodium lauryl ether sulfate (SLES), which heavily degrease the skin and hair; and are combined with amphoteric surfactants betaine or sulfobetaine, as a secondary surfactants, which act as foam stabilizers, solubilizers and/or thickeners. Prolonged and repeated application of these agents changes the natural pH of the skin from slightly acidic (pH=4.5-6) to neutral and alkaline values, which corresponds to reproduction of the pathogenic microorganism.

Also, the corneal layer disrupts the ratio of proteins and structural lipids that provide a stable skin barrier function. The hydrolipidic film recovers very quickly, but long-term damage to the skin's barrier function occurs after repeated skin exposure to these SAAs and its cumulative. These disorders in the structure and function of the skin are manifested in the form of different skin conditions (dry skin, cumulative irritation, irritant and allergic dermatitis) (Pedersen et al., 2004).

Materials and methods

The declaration of composition of 10 types of foreign and 10 types of domestic production shampoos on the market of the Republic of Serbia was analyzed. The types and amounts of surfactants present in the basic role of shampoo were analyzed, with an emphasis on determining the presence of substances that have the potential to exhibit skin irritant or allergic reactions.

Results and discussion

SLS and SLES (about 30%) are most often represented as primary surfactants in shampoos both, domestic and foreign in Serbia. These are preparations where, in addition to the content of SAAs (5-15%), they are also indicated which are
SAAs used. The most common amphoteric SAA in shampoos is cocoamidopropylbetain (about 7%), as a secondary surfactants as well as for reduction of the irritant effects of anionic SAAs.

In a large number of different studies, in addition to SLS and SLES, cocoamidopropylbetain, most frequently appears as a potential sensitizer or skin irritant (Goosens, 2005; Mangano et al., 2009). The effect of SLS on transepidermal water loss (TEWL) was also examined in relation to differences in age of subjects. A statistically significant increase in TEWL was measured in the younger group of subjects compared to the older group (Marrakchi & Maibach, 2006).

Some of occupational disease guides, indicate the possibility of developing skin irritation or skin allergic reactions in professions such as hairdressers and beauticians, who come into contact with these two potential irritants daily (Bogadi et al., 2014). Cleaning the skin with alkaline soaps and bath preparations results in cumulative irritation. Usually irritant reactions are local, occur after 24 hours at the site of administration, and they are limited. The skin tests for the allergen identification are either negative or indicate irritating dermatitis (Goosens, 2005; Pederson et al., 2004).

The irritation potential of SLES or SLS derives from their ability to remove hydrolipid layer from surface excessively. The irritation potential of cocoamidopropylbetain comes from the presence of impurities in the form of amines capable of producing nitrosoamines in the skin (Jessop, 2015). Combination of two irritants, as confirmed by several studies, results in increased penetration and interreaction with structures in the skin, resulting in damage to the barrier function and it is possible to manifest irritating dermatitis (Pedersen et al., 2004).

A comprehensive analyzes of different type of SLS toxicity due to different routes of administration, give a positive judgment about safety of use of SLS, with precautions for persons prone to skin irritation or allergic reactions (Bondi et al., 2015).

Conclusion

The effects of combination of allergens and irritants, when compared to the response to the same substances alone, are very different. The effects of exposure to multiple allergens or irritants must be thoroughly investigated and documented.

The results of such studies would be of great importance for the safe application of shampoos. The declarations of these preparations should include information about possible effects of combination of irritants found in these preparations. It can be an important aspect in the prevention of contact and irritant dermatitis.

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The presence and type of SAAs in shampoo-type preparations

Jasmina Bašić¹*, Isidora Milanović¹, Danijela Pecarski¹, Dragana Dragaš Milovanović¹

¹Belgrade Academy of Professional Studies, Department Medical College of Professional Health Studies, Cara Dušana 254, Zemun, Serbia

Introduction

SAAs (Surface Active Agents), surfactants or tensides, are amphiphilic substances of organic nature, which have the ability to be absorbed on the surface of particles, changing their surface tension. Thanks to their amphiphilic, polar-nonpolar dual nature, one part of their molecule is soluble in a particular liquid (the lyophilic part), whereas the other part is not soluble (the lyophobic part). The polar part of a molecule is called the head and is hydrophilic, whereas the nonpolar part is the hydrophobic tail soluble in fats. SAAs are substances present in washing agents, face and body care products, medications and para-pharmaceuticals, food products, as well as textile, paint and oil production, and in many other products. Those SAAs have different roles: moisturizers, emulsifiers, solubilizers, foamers/antifoamers, rheologic modifiers, antistatic agents, lubricants (Schramm et al., 2003) so, they have a role of a detergent in cosmetic preparation (shampoos, liquid soaps); in cosmetic emulsions (lotions and creams), they have a role of an emulsifier; in toothpastes, gels, lotions, solutions, they have a role of solubilizers or moisturizers.

Cleanliness maintenance products primarily have a task to clean the skin and hair from impurities, natural secretes (sebum and sweat), dead cells and the largest number of microorganisms. We need to be very cautious because of potentially damaging the hydrolipid film of the epidermis due to an excessive use of washing agents. The shampoo acting mechanism implies the creation of micelles in water, which have an affinity for fat and oil components which also include different impurities on the skin and hair, sebum included. The long-term and excessive use of these agents may lead to the drying-out of the skin and hair; the natural pH of skin changes, and the lipid/protein ratio in the horny layer is disturbed by the loss of the fats which have a protective role.

Materials and methods

In the research, an analysis of the chemical composition of 10 types of the shampoo of foreign production and 10 types of the shampoo of the domestic production (Serbia) was carried out, and a review of the type and amount of the present surface active agents in the shampoos was made with a reference to the safety of their application and potential adverse reactions.

Results and discussion

In the analyzed shampoo-type preparations, two types of ingredients are differentiated: SAAs (10-30%) with the HLB value from 13 to 15, and different additive types. In shampoos the basic or
primary SAAs are anionic, which serve to wash the vertex and hair, and auxiliary or secondary are amphoteric and nonionic, which improve the characteristics of the basic ones.

Topologically, SAA molecules consist of the compact polar head and the flexible nonpolar tail, which create micelles in a water solution in the self-aggregation process. Heads have a strong affinity for polar solubilizers, particularly in water (those are hydrophilic parts), whereas the nonpolar part of the molecule is a hydrophobic or lipophilic part soluble in fats. The polar group is represented by a heteroatom: oxygen, nitrogen, sulfur, phosphorus in the composition of the alcoholic, thiol, etheric, amino-, carboxylic, ester, sulfite, phosphate functional groups. A hydrocarbon branched or linear alkyl (alkylbenzene) chain 8-18 C atoms in length, which can be substituted by the atoms of halogen represents the hydrophobic part of the molecule. (Salager, 2002) The choice of a polar group (ionic or nonionic), as well as the relative size of the polar head and the nonpolar tail, is significantly determined by the physical-chemical characteristics of tensides (Mandavi et al., 2008). Physicochemical characteristics are also determined by the degree of the branching of the chain and the position of the polar group in the molecule of a surfactant. (Holmberg et al., 2002).

Anionic tensides have a role to create a foam and remove impurities. Most frequently and in the greatest percentage, we found alkyl-sulfates and alkyl-ether-sulfates, in the shampoos. Besides, there are also amidetersulfates, methyl-taurides and protein condensation products. Sodium-lauryl-sulfate, sodium-xylene-sulfonate, sodium-laureth-sulfate and sodium-lauryl-ether-sulfate are the most present in the analyzed shampoos.

Bside anionic tensides, there are also amphoteric, whose representatives are the four betaine groups: miranols (imidazole derivatives), N-alkyl-beta-amino-propionates, N-alkyl-beta-imidipropionates and betaines with amides of long-chain acids. Cocamidopropyl betaine is most present in shampoos. Amphoteric tensides are known to have bactericidal characteristics and mildly act on the skin of the head, alleviating the irritating action of anionic SAAs. Coconut acid diethanolamide is most frequently found in the shampoos. Their significant role also implies the alleviation of the irritation caused by anionic tensides. The anionic surfactant group is most and in the greatest percentage present in shampoos because of its detergent features and good solubility in water. They are stable in an alkaline environment and are not compatible with cations because of the formation of complexes and a reduction in the surface activity. They are economically cost-effective, and their safety for humans and animals has been researched by the Cosmetic Ingredient Review.

**Conclusion**

Because of the diverse applications of SAAs, their distribution into the living environment is great. There is an aspiration to formulate “greener” SAAs, which will have a minimal influence on the living environment, low toxicity and ecological justification. New, green surfactants should be of a structure which will not be any threat to the health of users, without the mutagenic and carcinogenic potentials and a tendency to develop toxicity (Jessop et al., 2015).

**References**


The use of dendrimers as a modern drug delivery platform

Elena Drakalska Sersemova¹*, Tanja Vasileva², Bistra Angelovska¹, Dijana Miceva¹, Natasha Miteva¹

¹Faculty of Medical Sciences, University “Goce Delcev” – Stip, Krste Misirkov 10-A, 2000 Stip, N. Macedonia
²Oaza Alkaloidi, Gladno Pole, Village Tarinci, Municipality Karbinci 2207, N. Macedonia

Introduction

The therapeutic potential of many active substances cannot be realized in clinical practice due to adverse physic–chemical properties, variable pharmacokinetics and a range of adverse effects causing low bioavailability and unsatisfactory therapeutic concentration in the target tissue. In order to overcome these problems, in recent years the emphasis has been placed on the study and characterization of different types of nanoparticles that offer many advantages over conventional therapy. The most common investigated and characterized nanosystems are dendrimers, as a new class of polymers.

Dendrimers as nanosystems are formulated from materials that are biocompatible, biodegradable and non-immunogenic. They have unique properties like thermodynamic stability, small particle size (1-20 nm), low polydispersity index and negative zeta potential (Svanson et al., 2018). The dendrimer structure is compatible with a range of hydrophilic and hydrophobic active substances with high encapsulation efficiency using different preparation methods (Abasi et al., 2014).

There are a several types of used dendrimers but PAMAM dendrimers are the most commonly studied dendrimers due to documented safety studies, and are candidates for encapsulating a large number of antineoplastic agents (Dubey et al., 2019).

The therapeutic efficacy of many antineoplastics is limited due to their poor penetration into tumor tissue and serious adverse effects on healthy cells. This section discusses the results of clinical trials incorporating the most commonly used antineoplastic drugs in dendrimer formulations (Mariyam et al., 2018).

Materials and methods

The propose of this paper is to review the structure of the dendrimers and the role of the components in their structure as well as compare and detect the factors which affect the stability of dendrimers and developing different formulation methods. We did a research for characterization of dendrimers with encapsulated active substance and processing and comparison of clinical trial results on the efficacy and toxicity of drugs incorporated into dendrimers against different types of disease.

Our research is made for characterization of incorporating the most commonly used antineoplastics drugs (doxorubicin, paclitaxel and docetaxel, camptothecin) in dendrimer formulations.

To accomplish these goals, we used data from relevant literature sources from primary, secondary and tertiary literature, with emphasis on original scientific research on the characterization and evaluation of dendrimers with incorporated active substances and processing of their results. We systematized the summarized literary data according

* elena.drakalska@ugd.edu.mk
to the actual treatment of the problem, noted the formulation aspects of using different methods and active substances, and identified the advantages and disadvantages by highlighting the possibilities of optimizing the therapy of various diseases of the dendrimer. We discussed the processed results, tabulated the patented products, and drew appropriate conclusions from the evaluated results.

Results and discussion

Doxorubicin is used in the treatment of several types of cancers, but due to the adverse effects, several formulations of doxorubicin with dendrimers have been investigated, and the results have shown that P1 and P2 particles inhibit cancer growth twice as much, and that is result of the additive effect of complex of dendrimers with DOX, as well as a significant reduction in side effects (Zhang et al., 2018).

PAMAM dendrimers have been proven to be ideal platforms for delivering also paclitaxel and docetaxel, significantly increasing water solubility and providing higher bioavailability (Li et al., 2018). Encapsulation of camptothecin resulted in a significantly higher percentage of accumulation in malignant cells compared to the free drug (Thiagarajan et al., 2010).

In addition to the widespread use of antineoplastic delivery, numerous clinical studies have shown that dendrimers, especially third- and fifth-generation PAMAM, are promising candidates for transdermal, oral and ocular delivery of numerous active substances with adverse pharmacokinetic properties. It is possible to modify the membrane with specific ligands that will provide targeted delivery to certain tumor cells with overexpressing receptors that will allow controlled release of the active substance into the malignant cell without affecting the surrounding tissues (Wolinsky et al., 2018).

Conclusion

Dendrimers are a challenge in modern therapy given the fact that they provide targeted delivery of drugs that can increase the bioavailability of the encapsulated drug and enable controlled and prolonged therapeutic activity.

The high cost, low solubility, volatility at certain pH values and short half-life make the application of other nanoparticles significantly difficult. Dendrimers provide controlled delivery of different types of active substances followed by increased therapeutic effect, achieving high concentration in the target cell and reducing the adverse effects.

From the processed data, we concluded that dendrimers are optimal carriers for many active substances providing higher solubility, greater stability and improved bioavailability, especially at antineoplastic treatment.

References


Effect of irradiation on the physicochemical and biopharmaceutical properties of Temozolomide loaded carbon nanotubes

Radmila Ilijeva1*, Anita Grozdanov2, Piotr Ułański3, Slawomir Kladubowski3, Marija Runceva1, Ana Ivceska1, Kristina Mladenovska1

1Faculty of Pharmacy, Ss. Cyril and Methodius University, 1000 Skopje, N. Macedonia
2Faculty of Technology and Metallurgy, Ss. Cyril and Methodius University, 1000 Skopje, N. Macedonia
3Institute of Applied Radiation Chemistry, Lodz University of Technology, Lodz, Poland

Introduction

The distinct properties of carbon nanotubes (CNTs), in particular high surface area (that provides high drug loading), intrinsic optical and thermal properties (that enable multimodal real-time tracking and photo-thermal applications), propensity to functional modification and biocompatibility and nanofluid nature make them useful for controlled brain (tumor) drug delivery. By attachment of reactive functional groups, basic electrical, optical and physicochemical properties can be changed and conjugation of ligands and drugs to the surface of CNTs enhanced. Their capacity to cross the blood-brain-(tumor)-barrier by energy-dependent and independent mechanisms (needle-like crossing) is another major advantage. Due to the electrical conductive capacity, strong mechanical properties and morphology similar to those of neurons, CNTs satisfy the requirements for successful neuro-restoration, genesis and plasticity. Also, the structure and dimensions of CNTs resemble those of certain neuronal structures and elements relevant for physiological activity and processing of neuronal information (Guo et al., 2017). Having all this in regard, the aim of the study was to prepare and characterize Temozolomide (Tem) loaded multi-walled CNTs (MWCNTs-Tem). In addition, the effect of irradiation on physicochemical and biopharmaceutical properties of MWCNTs-Tem (I-MWCNTs-Tem) was evaluated. Tem is a first line alkylating and radio-sensitizing agent used for treatment of anaplastic astrocytoma and glioblastoma multiforme (GBM).

Materials and Methods

MWCNTs were prepared by an original procedure of reduction of Li molten salts on graphite cathode (Dimitrov et al., 2013). Solution of Tem was added to MWCNTs suspension in 0,1M HCL (MWCNTs:Tem 1:3), ultrasonicated for 1h and stirred for 96h. Afterwards, the MWCNTs-Tem were isolated by ultracentrifugation (12000 rpm/min, 15 min, Eppendorf MiniSpin, Germany), rinsed 3× with double-distilled water and dried at room temperature. Encapsulation efficacy (EE) was determined as a difference between the total amount of Tem in the initial solution and filtrate. The drug content (DC) was calculated as a ratio between the total amount of loaded Tem and MWCNTs-Tem. The concentration of Tem was measured with

* radmila.ilijeva@gmail.com
UV/VIS spectrometer (λ=328 nm, UV/VIS Perkin Elmer Lambda 16, USA). Particle size distribution and zeta potential were determined by NanoZS-100 (Malvern Instruments Ltd., UK), after dilution of (I-) MWCNTs-Tem in PBS, pH 7.4 (0.0001M) with Polysorbate® 20 (2% (w/w), Sigma-Aldrich, UK). In vitro Tem release from (I-)MWCNTs-Tem was followed through a dialysis membrane (Mw cut-off 12000, Sigma-Aldrich) in PBS pH 7.4 (37°C±2°C, 50 rpm/min). At predetermined time intervals, samples were collected and concentration of released Tem was estimated. The release data were fitted into various models to determine the release kinetics. To find out the mechanism of drug release, 60% of drug release data was fitted in the Korsmeyer-Peppas (K-P) model. Samples have been irradiated by electron beam generated by ELU-6 linear accelerator (Elektronika, Russia), the absorbed dose being 50 kGy. Pulses of 6 MeV electrons (pulse duration: 4 ms) have been delivered at a frequency of 20 Hz, leading to an average dose rate of 5 kGy/min (as determined by alanine dosimetry).

Results and Discussion

EE of MWCNTs-Tem was 62.0±3.5% (n=6), while the DC 16.0±1.2% (n=6, theor. content 25%). Statistically significant differences were observed between the MWCNTs-Tem and I-MWCNTs-Tem in surface charge (-13.3±2.3 mV and -1.2±0.3 mV, accordingly, n=6) and d50/PDI (145/0.385 and 654/0.628, accordingly, n=6), suggesting more homogeneous size distribution of MWCNTs-Tem and formation of aggregates of I-MWCNTs that affect surface charge. Literature data point that exposure of MWCNTs on gamma-irradiation at lower doses (25 and 50 kGy) improved the graphitic order of MWCNTs, higher doses (100 and 150 kGy) introduced structural imperfection and at very high dose (200 kGy), the structure become distorted (Silambarasan et al., 2017). In a study of Jun et al. (2010), increased release rate of cancer ancillary drug tea polyphenols from chitosan-MWCNTs was observed by gamma irradiation. The opposite was observed in this study, where statistically significant difference in Tem release kinetics between MWCNTs-Tem and I-MWCNTs-Tem was observed. The cumulative percent of Tem released after 2 h and 9 days was higher (22±6% and 85±5%) from MWCNTs-Tem than I-MWCNTs-Tem (15±2% and 58±16% (n=3), accordingly), which can be explained by higher particle size of I-MWCNTs-Tem. Higuchi model was the best fit model for the Tem release (R=0.926 and R=0.957, k=4.04 h⁻¹ and 2.53 h⁻¹, accordingly). The exponent “n” in the K-P model was ≤ 0.45 for both (I-) MWCNTs-Tem, indicating Fickian diffusion in the drug release.

Conclusion

The paper demonstrates the potential of MWCNTs for delivery of Tem to tumor cells (incl. brain tumors), based on the basic physicochemical and biopharmaceutical properties. Incorporation of Tem into MWCNTs is simple and the content of Tem in the prepared MWCNTs is relatively high. The size of MWCNT-Tem is suitable for delivery via the BB(T)B, although there are literature data suggesting that cell accumulation is non-dependent on length/size of the CNTs (SWCNT and MWCNT with average size between 195 and 630 nm). Surface charge is also suitable for effective delivery in tumor cells, having in regard that slightly negative or neutral charge is required in central circulation. Tem showed low potential for early release from MWCNTs (and thus low potential for systemic toxicity) and potential for continuing release at the site of action, having in regard the pH of brain tumor cells (e.g. pH of GBM ~ 7.2). The exposure to irradiation has affected the physicochemical and biopharmaceutical properties of MWCNTs-Tem.

References


Cross contamination control strategy in multiproduct pharmaceutical manufacturing facilities

Viktorija Velkovska*, Meri Davcheva, Milkica Gligorova

Alkaloid AD, Blvd. A. Makedonski 12, 1000 Skopje, Republic of North Macedonia

Introduction

Multi product pharmaceutical manufacturing facilities offer flexibility, efficiency and cost reductions, because the same equipment is used for manufacturing different products. But such multiproduct manufacturing brings the risk of cross contamination. The term contamination means undesired introduction of impurities (chemical/microbial/foreign matter into or on to starting material or intermediate – during sampling, production, packaging or repackaging. While cross contamination is the contamination of a starting material, intermediate product or finished product with another starting material, intermediate product or finished product. One of the greatest challenges in multi-product facilities is the prevention of cross contamination.

The aim of this paper is to present the most effective strategies for cross contamination control in multi product pharmaceutical manufacturing facilities.

Cross contamination control strategies

A contamination control strategy should be implemented across the facility in order to assess effective prevention of cross contamination. The most effective ways to prevent cross contamination are described below.

Well-designed and operated facilities - Cross-contamination should be prevented for all products by appropriate design and operation of manufacturing facilities. Production premises should be of suitable size (adequate space for orderly placement of equipment and production materials to prevent mix-ups and contamination), construction and location. The facility must have smooth surfaces, so it can be reduced accumulation of dust and should permit easy cleaning and maintaining. Airlocks should prevent particulate contamination of the different areas. The opening of more than one door at a time should be prevented with proper door interlock systems. The flow of materials and personnel through facilities should be well defined and designed in such way to prevent mix-ups or contamination (European Commission, Guidelines for Good Manufacturing Practice, 2014).

Personnel - Only well trained, suitably qualified and authorized personnel should have access into production, storage and product control areas. Personnel should be well trained for the specific manufacturing technologies used in the production processes, then for cleanroom practices, contamination control and performing cleaning. Personnel should wear appropriate protective equipment and clean body coverings. It should be avoided direct contact between operator’s hands and starting materials, intermediate or bulk products, and also primary packing materials. Movement in clean areas, should be kept to a minimum and well controlled and defined (Pharmaceutical Inspection Convention Guide to GMP, 2018).

* vzdravkovska@alkaloid.com.mk
**Equipment** - Manufacturing equipment should be designed so that it can be easily and thoroughly cleaned. There should be written standard operating procedure for cleaning of each equipment. The cleaning process should be validated. If the equipment is difficult to clean, then it should be considered dedicating the equipment for manufacture of a single formulation of product. It is important that all the equipment, material used and the facilities should have cleanliness status labels. For every cleaning procedure should have records and should be performed checks in between batches. Where applicable should be used closed systems in production, so it can be prevented generation of dust and direct exposure of the product to the surrounding environment will be avoided, according to European Commission Guidelines for Good Manufacturing Practice (2014).

**Validated cleaning procedures** - Cleaning validation is a documented evidence that the cleaning procedure provides reproductive removal and cleaning of the previous product or detergents used for cleaning the equipment. Good cleaning procedures are fundamental. Manual cleaning is very subjective, as a result of operator-to-operator variability in the degree of cleaning. So the standard operative procedures for manual cleaning must include very detailed instructions for the cleaning procedures and the operators should be well trained for the cleaning procedure, to accomplish reproducible manual cleaning of the equipment. Cleaning procedures should be monitored at appropriate intervals after validation to ensure that these procedures are effective when used during routine production (Alley et al., 2017).

**Heating, Ventilation and Air Conditioning (HVAC systems)** - HVAC systems control airborne particles, dust and microorganisms through air filtration using high efficiency particulate air (HEPA) filters. Air must be controlled where product is exposed to the environment. The filtered air entering a production room can be turbulent or unidirectional (laminar). Laminar flow should be used in areas where there is excessive dust formation. The second important function of HVAC systems is maintaining room pressure. Clean areas must have higher room pressure than the surrounding areas. This means that when the door of the clean area is opened the air flow will be from the “cleaner” area towards the surrounding areas. Pressure control devices should be linked to an alarm system set which will indicate reduction of pressure differentials below set limits, according to World Health Organization Guidelines on HVAC systems (WHO, 2019).

Additional measure to maintain the pressure differentials that should be taken in consideration is interlock door system that do not allow simultaneous opening of the doors in the production area.

**Conclusion**

Controlling of cross contamination in multi product pharmaceutical manufacturing facilities is very important. Cross-contaminated pharmaceutical products can be life-threatening for the patients. There are many cross contamination control strategies that should be implemented to prevent cross contamination: well-designed facilities, well-trained operators; having written standard operating procedures for handling and storing material and products, for cleaning of equipment and facilities; well designed and maintained HVAC systems; validated cleaning and decontamination procedures; preventing generation and dissemination of dust; using cleanliness status labels on equipment.

**References**


Critical process parameters during semisolid manufacturing

Viktorija Veljanoska¹*, Elena Tomovska², Milkica Gligorova³

Alkaloid AD, Blvd. A. Makedonski 12, 1000 Skopje, Republic of North Macedonia

Introduction

Semisolid dosage forms are very significant part of pharmaceutical dosage forms. They are advantageous in terms of their easy application, rapid formulation, and ability to topically deliver a wide variety of drug molecules.

Topical semisolid dosage forms are normally presented in the form of creams, gels, ointments or pastes. They contain one or more active ingredients dissolved or uniformly dispersed in a suitable base and any suitable excipients such as emulsifiers, viscosity increasing agents, anti-microbial agents, antioxidants or stabilizing agents (Lachman et al., 1991). The objective of this paper is to provide a clear and depth knowledge about various methods, critical process parameters and strategies for the manufacturing processes of semisolid dosage forms.

Effect of raw materials on product characteristics

A wide range of raw materials is available for the preparation of a semisolid dosage form. The choice of adequate raw materials for a formulation development is made on the basis of the drug delivery requirements and the particular need to impart sufficient emolliency or other quasi-medicinal qualities in the formulation.

Semisolid dosage forms are complex formulations having complex structural elements. Mostly they are composed of two phases, one of which is a continuous (external) phase, and the other of which is a dispersed (internal) phase. The active ingredient is often dissolved in one phase, but sometimes when the drug is not fully soluble is dispersed in one or both phases, creating a three-phase system. The physical properties of the dosage form depend upon various factors, including the size of the dispersed particles, the interfacial tension between the phases, the partition coefficient of the active ingredient between the phases and the rheology of the system. These factors combine to determine the release characteristics of the drug, as well as other characteristics, just like viscosity.

Critical process parameters

There are several factors that effect on product characteristics and quality such as range of temperature during production process (heating and cooling), mixing methods and speeds, pressure and usage of additional tools such as pumps.

Temperature - Range of temperature during the process is critical for successful manufacturing. Very high values can cause chemical degradation and high concentrations of degradation products which will effect on product quality and stability. Insufficient heat can lead to batch failures. Fast cooling can result with precipitation of solubilized ingredients. This step is very critical especially during emulsification step of oil-in-water emulsion. If the temperature of the water phase is much cooler than that of the oil phase, the melted constituents of the oil phase may solidify upon introduction into the aqueous phase and never properly form the emulsion, possibly even resulting in solid matter in

* vveljanoska@alkaloid.com.mk
the batch. Heating too slowly can result in poor yields from evaporative loss. Heating too rapidly may burn areas of the batch in contact with the heating surface and the risk of presence of burnt material in the batch will be high. Rapid cooling can result in precipitation or increased viscosity of the product. The successful consistency of semisolids depends from proper rates of heating and cooling (Tanesh et al., 2016).

**Shearing and mixing** - Second very important factor that has effect on product quality is determination of type of shear and the optimal mixing times, methods and speeds. Process like emulsification requires high shear mixing or homogenization for obtaining the desired droplet size and dispersion, but there’s also processes like gel formation which require low shear in order to achieve optimal physical characteristics (like viscosity of the product). Proper mixing speeds must be obtained for each phase (Langley et al., 2019). Optimal hydration depends on the amount of shear imparted to initially disperse the polymer into the medium. If the process involves only very low shear mixing, a polymer may never be completely dispersed and hydrated, which may result inadequate viscosity. Equipment, recirculation loop with pump, may also be used to correct uniformity without changing mixing speed or time. Proper mixing speeds for each phase at every batch is very important for achieving desired characteristics of the formulation and high quality product (Nwoko et al., 2014).

Optimizing mixing time requires identifying the minimum time required for ingredients to dissolve and the maximum mixing time before product failure. For polymeric gels over-mixing, especially high shear, can break down the polymer's structure. In emulsion formulations over-mixing may result with prematurely separation of the phases which will cost with drastic changes in viscosity (Nwoko et al., 2014).

**Flow rate** - Flow rates are the third very important thing during manufacturing of semisolid dosage forms. Optimizing flow rate involves determining the amount of shear or throughput needed. Water-in oil emulsions require a slower addition speed than a traditional, oil-in-water emulsion, and the flow rate in this kind of formulations must be modified appropriately. Using of pumps during the process should be with very big attention because can result with changes in product characteristics. Overhearing can occur if the formulation is pumped too quickly. If pumping is too slow, the formulation will experience extra time in an in-line homogenizer, thus also exposing the formulation to additional shear. Two processes that require experimentation to optimize flow rates are the use of a powder ejection system and an in-line homogenizer. Raw material dispersers and in-line homogenizers require proper flow rates for optimal usage. If the product is not flowing through a disperser at the proper rate, there will not be enough suction for properly incorporating the powders.

Monitoring the flow rate when using an in-line homogenizer is necessary in order to calculate the theoretical number of times the product passes through it (Nwoko et al., 2014).

**Conclusion**

Manufacturing process for production of semisolid dosage forms is very important step for ensuring production of high quality products which will be present on the market. The quality of the product depends from many factors but mostly from the characteristics of the raw materials, equipment and their performance and critical process parameters such as temperature, pressure/ vacuum, share speed and mixing and usage of additional parts as pumps. All these parameters should be monitored during production process in defined ranges. Only fully controlled process can result with manufacturing a high quality products according the specifications.

**References**


An introduction of a new generation of Proticles

Katja Fresacher¹*, Bettina Huemer¹, Martin Reiser¹, Andreas Zimmer¹

Institute of Pharmaceutical Sciences, Department of Pharmaceutical Technology and Biopharmacy, Karl-Franzens-University Graz, Universitätsplatz 1, 8010 Graz, Austria

Introduction

MicroRNAs (miRs) used as active ingredients were put more often into spotlight these days. In their application field, of course, there have to be some obstacles to overcome: On one hand there is the low bioavailability and cellular uptake and on the other hand affinity for enzymatic degradation (Blanco et al., 2015). Therefore, protamine-oligonucleotide-nanoparticles, so-called Proticles, basically consisting of miRs and protamine, a cationic peptide, represent a new generation of nanoparticulate (NP) Drug Delivery Systems (DDS) to deal with that challenges (Scheicher et al., 2015). Proticles are formed by a self-assembling mechanism due to electrostatic interactions between the positively charged protamine and the negatively charged miR (Junghans et al., 2000). To mediate the miR release profile and decrease the strength of the electrostatic interactions the strategy of functionalizing NPs with citric acid (CA) was investigated.

Materials and methods

Materials

miR miRIDIAN™ microRNA mimic negative control was purchased from Dharmacon (Lafayette, Colorado), protamine (free base) from Sigma Aldrich (St. Louis, Missouri) and citric acid from Caesar & Loretz GmbH (Hilden, Germany).

Nanoparticle preparation

All NPs were prepared in aqueous solutions by mixing miR and protamine, final miR concentration was 50 µg/mL. The mass ratio of miR and protamine (1:3) was determined experimentally (data not shown). Aqueous CA solution was added to the protamine solution in molar ratios from 1:1 to 1:20 before it was put to the miR solution.

Nanoparticle characterization

By using photon correlation spectroscopy (PCS) (Zetasizer Nano Series, Malvern Instruments, Herrenberg, Germany) the physicochemical properties of the different NP formulations were determined. Results are expressed as hydrodynamic particle diameter (Z-average), polydispersity index (PDI), in correlation to particle size distribution (PSD), and the surface charge as Zetapotential, which is an indicator for NP stability.

For further stability studies we developed a stability assay to determine the decrease in stability by adding 50 µL 0.01 M NaOH to the NP solutions and detect the total number of remaining NPs afterwards with PCS. The Proticle dispersions were prepared as described before and afterwards treated with 0.01 M NaOH and incubated for 1 h in a thermomixer (60 °C; 1000 rpm) before the assay.

Drug loading

The drug loading efficiencies were determined by an indirect quantification method using reversed
phase high performance liquid chromatography (RP-HPLC; Agilent 1260 Infinity, Agilent technologies, Santa Clara, California). An Agilent PLRP-S 50 x 2.1 mm column (3 µm, 300 Å) was used at 80 °C. Mobile phase A was 0.1 M Triethylammonium acetate buffer (TEAA), whereas mobile phase B was acetonitrile. A gradient was used with a flowrate of 0.2 mL/min. It was starting with 5% B for 1 min and increased to 35% B in 7 min afterwards. For detection a diode array UV absorption detector at 254 nm was used. The NP dispersions were centrifuged for two hours at 4 °C and 20 000 rcf, 10 µL of the supernatant was injected for quantification. Bound miR was calculated as the difference between the amount of used miR and the detected miR in percentage.

Results and discussion

Nanoparticle characterization

The addition of CA to the Proticles had no influence on their size (~110 nm) except sample 1:5. It is believed that this molar ratio totally neutralizes the positive functions of protamine which lead to a kind of steady state. However, the PDIs (<0.2) represent monodisperse NP populations as well as stable DDSs. The Zetapotential of the NPs without any CA was 31.32±1.32 mV. By increasing the CA content, a decreasing trend was observed, which correlates with a decrease in stability. Interestingly, the result of the twentyfold surplus is increasing again which can be explained by rising protonation of the system. The detected derived count rate (dCR) of NP dispersions is expressed as percentage of remaining NPs after the treatment with 0.01 M NaOH. As a 100% reference the dCR of Proticles without any CA and an addition of H₂O instead of NaOH was taken. The results highlight a significant decrease (p<0.05) in number of NPs which correlates with a decrease in stability. These formulations, which contain a ten- or twentyfold surplus of CA represent the lowest results and therefore the biggest lost in stability (~94% loss). One possible explanation for this observation could be protonation. Based on high proton concentrations due to acid addition the NPs started to repulse each other and therefore the interaction between the NPs is lower than for NPs with less acid functions.

Drug loading

With a RP-HPLC system the drug load capacity of the Proticles was visualized and determined. Therefore, all tested formulations presented a drug load of >92%, no differences due to CA ratios could be found. These results give us important information about the preparation procedure – which seems to be well working - and the possible capacity of the advanced Proticles concerning drug load and transport efficacy. High binding affinities may help to improve a successful and sufficient dissociation rate of miR in further experiments.

Conclusion

The aim of this work was to characterize advanced Proticles, which basically consist of the positively charged protamine and a negatively charged miR supplemented with CA. The formation of NPs occurs immediately by mixing the protamine solution and the miR solution due to electrostatic interaction between the two components. We observed no difference in particle size, except NPs with a fivefold surplus of CA. A monodisperse PSD over all formulations was found. Due to increasing CA ratios the Zetapotential was decreasing, which lead to the assumption that higher CA contents destabilize the Proticles. These findings could be supported with the results of the self-developed NaOH assay. By applying NaOH to the NPs a statistically significant (p<0.05) stability reduction (up to ~94 %) was observed due to addition of CA.

A miR binding capacity of >92 % independent from CA ratios has been found, which demonstrated a well working and robust preparing procedure. All in all, this new approach of DDSs constitutes a very promising candidate to deal with the difficulties of miR drug delivery and cellular drug release.

References


Membrane interactions and cellular uptake of an amphipathic cell-penetrating peptide as a delivery system for miRNA

Ivana Ruseska¹*, Anna-Laurence Schachner-Nedherer², Andreas Zimmer¹

¹Institute of Pharmaceutical Sciences, Department of Pharmaceutical Technology and Biopharmacy, University of Graz, Universitätsplatz 1, 8010 Graz, Austria
²Division of Biophysics, Gottfried Schatz Research Center for Cell Signaling, Metabolism and Aging, Medical University of Graz, Neue Stiftungtalstraße 6, 8010 Graz, Austria

Introduction

Cell-penetrating peptides constitute a promising strategy for the intracellular delivery of therapeutic molecules, such as nucleic acids. The non-covalent approach based on the amphipathic N-TER peptide has shown to be successful in the intracellular delivery of miRNA-27a by inducing its antiadipogenic effect in 3T3-L1 cells. However, information regarding the events taking place at the cellular interface is lacking. Furthermore, information on the uptake process often comes as confusing and contradictive. The main focus of our study is to examine how the peptide interacts with the cell membrane, is this interaction fatal for the cells, or is it just the first step towards the peptide’s internalization into the cells?

Materials and methods

Materials

The N-TER peptide is part of the “N-TER Nanoparticle siRNA Transfection System” from Sigma Aldrich (Vienna, Austria). For the complexes, three different nucleic acids from Dharmacon were used (GE Healthcare, Vienna, Austria): double stranded miRNA mimic (miRNA-27a), miRNA mimic used as a non-targeting control (NTC) and miRNA mimic transfection control with Dy547 (Fluo-NTC). For in vitro experiments, mouse embryonic fibroblast-derived 3T3-L1 preadipocytes were used.

Sample preparation

To obtain standard N-TER peptide-nucleic acid complexes, aqueous solutions of the N-TER peptide and the respective nucleic acid (miRNA-27a, NTC or Fluo-NTC) were mixed in well-defined ratios reaching a final nucleic acid concentration of 650 nM. For obtaining concentrations suitable for in vitro studies, the standard complexes (650 nM) were diluted using low glucose DMEM.

Cell viability and cytotoxicity studies

3T3-L1 cells were seeded in 96-well plates with a seeding density of 8x10³ cells. Transfection was performed 24 h after seeding, by diluting the standard samples (650 nM) to final concentrations of 5, 10, 25, 50, 80, 100 and 160 nM, followed by incubation intervals of 4 and 8 h. LDH leakage from the cells was measured using the Promega

* ivana.ruseska@uni-graz.at
CytoToxONE™ assay (Promega, USA). LDH release from cells treated with 9% Triton X-100 in water was defined as 100% leakage. For the MTS assay, cells were treated similarly as they were for the LDH assay. Formazan production 4 h post transfection was measured using the CellTiter96®AQueous One Solution Cell Proliferation Assay (Promega, USA). Formazan production by untreated cells was defined as 100% cell viability.

Fluorescence imaging

3T3-L1 cells were seeded on glass bottom dishes and treated with the N-TER peptide alone, or complexed with Fluo-NTC (50 and 100 nM) for 4 h. Untreated cells served as control. Cells were then fixed with 3.7% formaldehyde and permeabilized with 0.1% Triton X-100. Alexa Fluor 488 Phalloidin was used to stain the actin cytoskeleton, and the nucleus was labeled with Hoechst. Images were obtained using CLSM (510 Meta, Carl Zeiss GmbH).

Cellular uptake studies

3T3-L1 cells were seeded in 96-well plates and transfected using sample concentrations of 5, 10, 25, 50, 80, 100 and 160 nM, followed by an incubation period of 2 h, at 4°C and 37 °C, with or without ATP-depleting agents (sodium azide and 2-deoxy-D-glucose). After 2 h, the cells were washed once using 100 µL PBS and then 100 µL of medium was added. Then, all the wells were lysed by 50 µL 0.5% Triton X-100 in 0.2 M NaOH. The cellular uptake was quantified by measuring the fluorescence intensity using a microplate reader.

Results and discussion

The aim of our study was to investigate the interactions that take place between the N-TER peptide, used as a delivery system for miRNA, and the cellular membrane, as well as to get some initial insight into its uptake. Using the self-assembly method, complexes between the N-TER peptide and the nucleic acids are formed due to electrostatic interactions. The resulting complexes have a size of 200-300 nm and are positively charged (Schachner-Nedherer et al., 2019).

The results obtained from the viability and cytotoxicity studies clearly indicate that the N-TER peptide has no membranolytic activity and the cells’ viability seems to be intact in all tested conditions. The previously described antiadipogenic effect of the “naked” peptide, which was assumed to be due to membrane perturbation or mechanical stress (Schachner-Nedherer et al., 2019), is most likely due to the peptide’s intrinsic ability to induce cytoskeletal rearrangement. In this way, the peptide increases the membrane fluidity and improves its own cellular uptake (Gerbal-Chaolin et al., 2007). This is in accordance with the images obtained using CLSM, where there is a significant difference in the cytoskeleton of cells treated either with the peptide alone, or complexed with Fluo-NTC, compared to the untreated cells. Furthermore, the conducted uptake studies show a small difference between the uptake at different temperatures, as well as after using ATP-depleting agents, which might indicate that the N-TER peptide leans toward direct translocation as a mode of entry into the cells, which is reasonable after the aforementioned increase in the cellular permeability.

Conclusion

In vitro studies using the 3T3-L1 cells are performed in order to investigate the interactions and the uptake of the N-TER peptide, as a delivery system for miRNA. The studies have shown that the peptide is safe and suitable as a delivery system. It has an intrinsic ability to influence the cytoskeletal network, increase the membrane’s permeability and thus, improve its own cellular uptake, most likely via direct translocation.

References

Pharmaceutical use of nanocellulose produced by enzymes

Patricia Leitner¹, Chao Zhong², Barbara Petschacher², Bernd Nidetzky²,³, Andreas Zimmer¹, Christina Petschacher¹*

¹Institute of Pharmaceutical Sciences, University of Graz, Universitätsplatz 1, 8010 Graz, Austria
²Institute of Biotechnology and Biochemical Engineering, Graz University of Technology, NAWI Graz, Petersgasse 12, 8010 Graz, Austria
³Austrian Centre of Industrial Biotechnology, Krenngasse 37, 8010 Graz, Austria

Introduction

Cellulose is an indispensable gelling agent in pharmaceutical hydrogels (HGs). There are two main strategies to gain cellulose: the top-down process from plants and the eco-friendly bottom-up synthesis out of single sugar molecules. By these methods celluloses of different degree of polymerization (DP) can be obtained. The production and use of short-chained cellulose (DP<50), called nanocellulose (NC), is a promising, rather new development. NC shows a high biocompatibility, liquid absorption capacity, porosity and mechanical strength. The latest development to achieve more defined NC products and controlled yield is an enzymatic bottom-up in-vitro production. Highlight of this technique is the possibility to produce soluble (DP ≤ 6) as well as insoluble (DP > 6) NC, the uniformity of DP, short production periods and defined modifications of the cellulose (Serizawa et al., 2017; Zhong et al., 2019).

HG prepared of insoluble, enzymatically synthesized NC, showing a self-assembled nanoribbon structure, bear new potentials for drug delivery. This work focused on the preparation and characterization of HG from enzymatically synthesized, insoluble NC with a DP of 7–10 and their use as drug delivery systems. To ensure purity and inertness of the NC, a process to remove possible production residues was established in a first step. After purification and freeze-drying of the NC, HGs were produced by addition of water. These HGs were intensively characterized by rheological measurements. Afterwards different active pharmaceutical ingredients (APIs) were successfully loaded into the HGs and their release was studied in permeation and diffusion tests.

Materials and methods

The studied NC was enzymatically synthesized under conditions described by Zhong et al. (2019) using cellobiose as primer. Unless stated, all materials used in this work were purchased from Merck KGaA (Darmstadt, Germany) or Carl Roth GmbH + Co. KG (Karlsruhe, Germany) in highest purity. All tests were at least duplicates.

Purification and freeze drying

For purification of the NC, four washing steps with acetic buffer pH 4.5 were performed at a mass ratio NC:buffer=1:1. After addition of buffer and short vortexing at 3000 rpm, the samples were centrifuged at room temperature (RT) and 14000 rpm. The purified NC pellet was frozen in liquid nitrogen and freeze-dried in a Lyovac GT2 (SRK Systemtechnik GmbH, Riedstadt, Germany).
Determination of removed phosphate and enzyme

The supernatants of the purification were subjected to a quantitative, colorimetric phosphate assay (Zhong et al., 2019). Quantification of the enzyme was done by Roti®-Quant. All photometric measurements were performed by a Nanophotometer Implen P-Class (Implen GmbH, München, Germany).

Hydrogel preparation and drug loading

The freeze-dried NC was reconstituted with MilliQ®-water to its original weight. For drug loading, 80% of the total water amount was added to the NC at first. The samples were vortexed and heated up to 95 °C for 5 minutes. The missing 20% of water, including gentamicin-sulfate or diclofenac-sodium purchased from G.L. Pharma GmbH (Lannach, Austria), were added and vortexed again. HGs containing 3 mg/mL gentamicin (GM) and 1% diclofenac-sodium, respectively, were achieved.

Rheological characterization

Rheological measurements were performed by a Physika MCR 301 (Anton Paar GmbH, Graz, Austria) using a cone-plate device (CP-25). Flow curves with increasing shear rates (0.01 – 2000 s\(^{-1}\)) were set up. Additionally, rotation and oscillation recovery tests after high shear load (2000 s\(^{-1}\)) were conducted.

Drug release

According the gentamicin gel, a diffusion test on Müller-Hinton agar plates was carried out using Staphylococcus epidermidis as test germ. For this test 3.33 mg gel (10 µg GM) and 10 mg gel (30 µg GM) were used. The drug permeation of the diclofenac gel was tested in Franz cells (PermeGear, Pennsylvania, USA) in combination with the hydrophilic-lipophilic multilayer membrane PermeaPad® (innoME GmbH, Espelkamp, Germany).

Results and discussion

The enzymatically synthesized NC was purified by several washing steps with acetic buffer to remove the by-product phosphate and the enzymes. While phosphate could be eliminated rather completely, only 50% of enzymes were washed off. After lyophilization of the insoluble NC, HGs were produced by addition of MilliQ®-water. Rheological measurements of these HGs showed a dynamic viscosity of about 600 Pa\(\cdot\)s at 0.01 s\(^{-1}\) and RT and revealed a pseudoplastic-thixotropic flow-behavior. As G’, representing the elastic portion of the gel, was higher than G’’, representing the viscous portion, before and directly after high shear load, the HG seemed to reconstruct quickly.

For inclusion of an API, the lyophilized NC was reconstituted with aqueous API-solutions including diclofenac-sodium and gentamicin-sulfate, respectively. During the drug loading heat of 95 °C was applied to inactivate the remaining 50% of enzymes. The API solution was taken up completely by the NC under swelling. The drug release of the gentamicin gel was successfully proven by diffusion tests. Staphylococcus epidermidis was not growing within an inhibition zone of at least 14 mm, proving a qualitative drug release and the activity of the antibiotic API. Referring to the diclofenac gel, the API-permeation tested in Franz cells reached 4% after 6 h. Additionally, a 1% diclofenac solution was tested. Within 6 h approximately 26% of the API permeated from the diclofenac solution through the membrane. This is a 6.5 times higher permeation rate than for the NC gel, indicating a retardation effect of the gel.

Conclusion

To the best of our knowledge, this is the first time an enzymatically synthesized NC was used for the preparation of drug-loaded hydrogels. The results shown are a promising starting point for further investigations of these NC hydrogels as drug delivery systems as they are capable of taking up APIs in therapeutic concentrations, show a pseudoplastic-thixotropic flow-behavior and a prolonged drug release after topical application.

References


Formulation development of prolonged-release matrix tablets - factors influencing drug dissolution rate

Oja Ali Memed1*, Maja Hadzieva-Gigovska1, Dejan Kunlesi1, Eleonora Trajanovska1, Packa Antovska1, Sonja Ugarkovic1, Maja Simonoska Crcarevska2, Marija Glavas Dodov2

1Research & Development, Alkaloid AD-Skopje, Blvd. Aleksandar Makedonski 12, 1000 Skopje, N. Macedonia
2Faculty of Pharmacy, Ss. Cyril and Methodius University, Majka Tereza 47, 1000 Skopje, N. Macedonia

Introduction

Chronic pain remains a major societal burden that is associated with a decline of normal daily functioning and quality of life. Appropriate management of chronic pain aims to improve quality of life and daily function by alleviating not only pain symptoms, but also comorbid conditions (Martin et al., 2016). Oral opioids have become the drugs of choice for the treatment of moderate-to-severe chronic pain because of flexibility, convenience and ability to maintain relatively steady blood concentrations (Petrovska Jovanovska et al., 2018). Controlled release formulations could be a suitable dosage forms in chronic pain management (i.e. reduced dose frequency, less fluctuation in plasma concentration, reduced side effects and good patients’ compliance).

For the design of generic oral drug product with prolonged release properties using Quality by Design, QbD approach, a variety of polymers with different physicochemical characteristics could be used in order to modulate the drug release behaviors. Therefore, during the one factor-at time experiments it is highly desirable to determine the critical material attributes (CMAs) of the selected excipients (controlled release polymer/s), to evaluate the transport mechanism involved in the drug release process, as well as to be able to predict quantitatively the resulting drug release kinetics as the product most important critical quality attributes (CQA) (Saurí et al., 2014).

The aim of this study was to develop a generic film-coated matrix tablets with water soluble opioid drug (API). In that direction, we have evaluated the influence of different types of polymers (HPMC, PVAc/PVP, HEC, PEO and PMAMMMA) on the properties of designed tablets in order to find the polymer or combination of polymers which will give most similar release profile with the reference drug product.

Materials and methods

Different formulations of film-coated tablets were prepared by wet granulation process using S1. HPMC (Colorcon, DE), S2. HEC (Ashland, UK), S3. PVAc/PVP (BTC, DE), S4. PEO (Colorcon, DE) and S5. PMAMMMA (Eudragit, DE) as drug release modifying polymers in concentration of 30% (w/w) respectively. The active substance (API, opioid analgesic, hydrochloride salt, BCS class II) was pre-blended with microcrystalline cellulose (FMC, IR) and selected polymer (S1-5) in high shear granulator.
Afterwards, granulation liquid was added, the wet mass was passed through 0.630 mm sieve and the granules were subsequently oven dried (MOV-212S; Panasonic, JP). The dried granules where passed through 0.813 mm sieve and blended with magnesium stearate (FaciSpa Carasco, IT). The final blends were subsequently compacted into round 7.0 mm tablets with compression force of 5.0-5.5 kN (Korsch XL 100, DE). Prepared tablet cores were coated with Opadry 20A220058 Yellow (Colorcon, DE), (O’HARA Labcoat M).

Final blends were characterized for bulk/tapped density, Carr-index, Hausner ratio and angle of repose according to Ph. Eur. 8.7 methods. Prepared film-coated tables were evaluated for mass and mass variation, hardness, thickness and diameter (TBH 425 TD, Erweka GmBh, DE). Prepared tablet cores were coated with Opadry 20A220058 Yellow (Colorcon, DE), (O’HARA Labcoat M).

In vitro drug release studies were performed for 12 h in 900mL simulated gastric fluid (Ph. Eur) as dissolution media maintained at 37±0.5 °C. Obtained dissolution profiles were compared with the reference drug product, according to the EMA guideline for bioequivalence (EMEA, 2010). To analyze the in vitro release data various kinetic models were used to describe the release kinetics.

Results and discussion

The Quality target product profile (QTPP) was set according to reference drug characteristics (round biconvex film–coated tablets with mass of 135.00 mg±7.5%, hardness 11–13 kP, diameter 7.00 mm±0.15 and thickness 3.1–3.6 mm) and appropriately justified in all segments. The final blends characterization showed differences in the flow properties of the granules, namely S1 and S2 had a fair flow, whiles S3-5 had acceptable flow properties. All manufactured blends, regardless their flow, were appropriately compressed and film-coated. Prepared film-coated tablets were smooth and elegant in appearance. The formulated tablets passed the uniformity of weight, uniformity of thickness and diameter tests respectively and were in acceptance criteria according to QTPP of the reference product, except for S3, were results for hardness of the tablets was in unsatisfactory level (2.3 kP). Obtained results from the in vitro release studies pointed the influence of the used polymers on API release behavior. The S4 and S5 showed significant differences with respect to the release rate of API compared to reference product (similarity factor $f_2$ of 34.64 and 14.93, respectively). On the other side, S1-3 had the $f_2$ of 53.5, 51.1 and 55.2, respectively, thus representing potential candidates for further formulation modification and evaluation. The drug release data from all examined samples fit well to the Higuchi expressions, which points that drug release mechanism independently from which polymer/polymers will be used, will be a complex mixture of diffusion, swelling and erosion.

Conclusion

Prolonged release film-coated tablets of water-soluble opioid analgesic have been successfully formulated using HPMC, HEC or PVAc/PVP as drug release modifiers. The type of polymer used as CMA was found to significantly affect the tablet properties, especially the API release rate, as the CQA of the final drug product and were able to provide the desired drug release over a 12 h time period.

References


Effect of variation of active ingredient particle size on the dimension of granules produced with high shear wet granulation process

Elizabeta Atanaskova*, Natasa Anevksa Stojanovska, Sonja Ugarkovic

Research and development department, Alkaloid AD, Aleksandar Makedonski 12, 1000 Skopje, N. Macedonia

Introduction

Granulation is a particle size enlargement process that converts fine or coarse particles into physically stronger and larger agglomerates. During the granulation, primary powder particles are made to adhere to form multiparticle entities called granules (Shinde et al., 2014). Resulting granules usually have larger particle size and bulk density compared with the starting material (Farag Badawy and Hussain, 2004). The physical properties of produced granules are mostly dependent on the characteristics of starting material particularly within the wet granulation process where particle interfaces are combined with the interaction of primary particles with granulation liquid.

According to Schaefer and Mathiesen (1996), there are two different mechanisms of granule nucleation during the wet granulation process in a high-shear mixer: distribution and immersion. Mechanism of distribution occurs when the particle size of molten binder droplets is smaller than the solid particles whereas the immersion occurs when the molten binder droplets are larger than solid particles for instance, for granulation where high viscosity binders are implemented. In case of the immersion mechanism of granulation, the primary solid particles are deployed around the surface of high viscosity binder's droplets. Consequently, a slight variation of primary solid particle dimensions will result in changes in the number of particles bind by the same quantity of high viscous binder solution, leading with incensement of un-granulated primary particles at the end of the granulation process.

The aim of this study is to present the differences in particle size distribution of granules (in high dose formulation) produced with high shear wet granulation process using high viscosity binder and three different grades of the active ingredient, all of them satisfying the specification requirements. It is anticipated that this information will be valuable for future prudent selection of an alternative active substance manufacturer during the product lifecycle.

Materials and methods

Materials

The materials used were of pharmaceutical grade and include Anhydrous colloidal silicon dioxide (Aerosil 200, Evonik Industries AG), Croscarmellose sodium (Ac-Di-Sol, DuPont Nutrition & Health), Mannitol (MERCK KGaA), Starch (Binder, BASF). Purified water was used for dispersion of starch and formation of starch paste as granulation liquid. The model drug substance X (from two different API manufacturers) was used in three diverse grades all of them satisfying the specification requirements: d50<20 μm, d90<80 μm.
The grades of model drug substance X used in the described experiments were: Grade I (d10=1.63 μm, d50=4.15 μm, d90=9.15 μm), Grade II (d10=3.68 μm, d50=12.6 μm, d90=38.1 μm) and Grade III (d10=4.50 μm, d50=21.75 μm, d90=78.75 μm).

**Characterization of active ingredient particle size**

The particle size of each grade drug substance X was determined by laser diffraction (Mastersizer S, Malvern Instruments Ltd., Worcestershire, UK) dry technique.

**Granulation experiments in the high shear mixer**

The granulation of the three different grades of the model drug substance X was performed in a laboratory-scale bottom-driven vertical high shear granulator with a horizontal chopper shaft (Diosna 4 L bowl) using a batch size of 1.0 kg.

All three trials were of the same composition, produced with the same process parameters, only the grade of the model drug substance X was varied. The formulation consisted of 97.70% active ingredient, 0.19% anhydrous colloidal silicon dioxide, 0.88% mannitol and 1.23% croscarmellose sodium. 10.43% starch paste was used as a binder.

Re-granulation of the wet granules was performed on Quadro Comil U5 laboratory scale conical mill. Wet granules were dried in Mycrolab fluid bed drier to a moisture content of <1.0 %.

**Granule size analysis**

The particle size distribution of the granules was determined according to the analytical sieves method from Ph. Eur, using Retsch analytical sieve-shaker AS 400 (Retsch GmbH) with ISO standard sieves.

The particle size distribution of the granules was described as a cumulative fraction under sieve. The granules from all trials were analyzed on a sieve-shaker using a series of sieves with different sizes (80 μm, 125 μm, 250 μm, 315 μm, 630 μm).

**Results and discussion**

A significant difference between the analyzed simples was obtained within cumulative fraction under sieve size 250 μm. Granules size was found to be smaller (Trial 3 - Cumulative fraction under sieve 250 μm = 85.5%) when the initial mean particle size of the active ingredient is bigger (grade III - mean particle size = 21.75 μm). Furthermore, the granules produced with a smaller initial particle size of active ingredient (grade I - mean particle size=4.15 μm) tent to form coarse granules (Trial 1 - Cumulative fraction under sieve 250 μm = 48.90%) after granulation with the same process parameters.

The particle size mean diameter of the active ingredient used in trial 2 was intermediate (mean particle size = 12.6 μm) compared to the other two active ingredient grades, consequently producing granules with medium particle size (Trial 2 - Cumulative fraction under sieve 250 μm = 62.80%).

The particle size of granules produced with high viscosity binder as starch paste appeared to be inversely related to the starting material mean particle size. During the wet granulation process with high viscosity binder, granule growth is greater if the initial active ingredient particle size is smaller.

**Conclusion**

Although, all grades of active ingredient used in this study complied with the specification requirements, changes within the approved particle size distribution criteria lead to the production of final granules with different particle sizes.

Experimental data obtained in this study contribute to the immersion mechanism of granule nucleation. When the droplets of the binder are bigger than the initial solid particle size, the number of particles able to be bounded with the binding material is rising if the mean particle size of the starting material is decreasing. The evaluated influence could be especially important within high dose formulations as the final product quality is particularly based on active ingredient properties.

**References**


Influence of sterilisation by irradiation on an antiviral eye ointment

Frosina Jovanovic*, Eleonora Trajanovska, Ana Atanasova, Emilia Arsovska Popovska, Monika Stojanovska Pecova, Jelena Dimitrovska, Suzan Memed Sejfulah, Packa Antovska, Sonja Ugarkovic

Research and Development, Alkaloid AD, Blvd. Aleksandar Makedonski 12, 1000 Skopje, North Macedonia

Introduction

Sterilisation by irradiation is becoming a commonly used method for sterilisation of pharmaceutical drug products that cannot be sterilised by other means such as sterilisation with dry heat, moist heat under pressure, gas, high intensity visible light or filtration. For semi-solid eye preparations filled in their final container sterilisation by irradiation can be considered as most suitable. (EMA, 2015; WHO, 2011)

In this study, we used an eye ointment in form of a suspension containing an antiviral agent as an active substance and white paraffin as an ointment base. Thermal sterilisation was not applicable considering the low melting point of the ointment base. Due to toxic residues problems or the high process costs, sterilisation with ethylene oxide was avoided. Sterilisation by irradiation was achieved by exposure of the product to gamma rays from cobalt 60 ($^{60}$Co) as an isotopic source, and to a beam of electrons energised by a suitable electron accelerator at the reference absorbed dose of 25 kGy. The procedure and precautions employed were such as to achieve sterility assurance level (SAL) equal to or less than $10^{-6}$ according to Ph. Eur. 5.1.1 “Methods of preparation of sterile products” and ISO standard 11137.

The aim of this study was to evaluate the influence of gamma and e-beam irradiation at a dose of 25 kGy when applied for the purpose of sterilisation of an antiviral eye ointment.

Materials and methods

An antiviral agent was used as an active substance and white paraffin was used as an ointment base. The manufacturing process and production equipment for the eye ointment included standard technological steps such as melting and mixing, dispersion of the active ingredient and homogenisation, cooling and filling. Sterilisation by irradiation was performed with an irradiator Gammatron 1500 with $^{60}$Co source type RSL 1800 and Rhodothron TT100-IBA-X, respectively for gamma and e-beam irradiation. The delivered doses were monitored using alanine dosimeters. Sterility tests of the samples were performed with Ph. Eur. Method 2.6.1. “Sterility”. The evaluation of the parameters, assay of active substance and the impurity profile, was done using a HPLC method. The particle size of the active substance in the eye ointment was monitored in accordance with Ph. Eur. monograph on semi-solid eye preparation (1163) using automated microscope Morphology G3. Viscosity analyses were performed using a

* fkralevska@alkaloid.com.mk
Brookfield DV2T RV viscometer with a T-bar helipath spindle.

Results and discussion

The antiviral eye ointment was evaluated for its predetermined critical quality attributes (CQAs) including sterility and physico-chemical properties such as assay of active substance, related and degradation products, viscosity and particle size. The results from the tested product parameters were compared before and after performing the sterilisation by two types of irradiation.

Sterility is a fundamental issue in manufacture and use of eye products. Performed sterility tests for samples after sterilisation by irradiation showed that the sterility of the antiviral eye ointment was achieved when the product was irradiated by both gamma and e-beam irradiation with a dose of 25 kGy.

The main concern regarding sterilisation by irradiation, besides sterility assurance, is that the process should not cause any significant changes in the quality of the pharmaceuticals, specifically, alteration in the content of active ingredient or increase of impurities. All results for the parameter assay of antiviral active substance after sterilisation by gamma and e-beam irradiation were within the established acceptance criteria and no decrease was observed compared to the initial values obtained before sterilisation. The impurity profiles were similar before and after sterilisation by gamma and e-beam irradiation. From the data obtained for specified impurities, unspecified impurities and total impurities, it was concluded that all results were below the established acceptance criteria and no increase due to radiolysis was observed. Conclusively, it is not expected that the related and degradation products of the tested eye ointment could impose upon the safe use of the product.

The particle size of the active substance is a critical parameter for eye ointments containing suspended particles of the active substance. This parameter can affect the safety of the product, since if particle size is not within the acceptance criteria it can cause irritability in the eye. For this reason, the particle size of the active substance in the eye ointment was controlled by use of micronized grade and monitored during different stages of development and manufacture of the finished product. No change of particle size was observed between all tested samples before and after the sterilisation.

Rheological properties of ointments have a significant role in terms of processing and application. The viscosity analyses before and after sterilisation for all tested samples show no significant change of viscosity, therefore, the sterilization has no impact on the homogeneity of the product and on the application from the container.

Conclusion

Based on the obtained results for evaluated CQAs and taking into consideration the product characteristics, sterilisation by gamma and e-beam irradiation at a dose of 25 kGy can be considered as suitable method for achieving sterility of the product. For both types of irradiation comparable effect on the quality of the antiviral eye ointment, regarding sterility and physico-chemical properties, was observed.

References

Optimization of tablet compressing process using experimental design approach

Katerina Tnokovska*, Krume Toshev, Bojana Trifunovska Vulovska, Packa Antovska, Sonja Ugarkovic

Research & Development, Alkaloid AD, Blvd. Aleksandar Makedonski 12, 1000 Skopje, R. N. Macedonia

Introduction

Identification of critical process parameters (CPPs) that can have an effect on product Critical Quality attributes (CQAs) and determining the functional relationships that link process parameters to product CQAs is critical step in understanding the process and process control (ICH guideline Q8).

According to the initial risk assessment, performed during formulation/process development, regarding the link between Critical Quality Attributes and Critical Process Parameters, related to the equipment connected with the technological process of the product containing BCS III active substance, tableting speed and compression force have been assessed as critical process parameters in tablet compressing process (Tho and Bauer-Brandl, 2011).

The aim of this study was to optimize the tablet compression process and to predict the ability of the process to produce product with quality attributes which are within a predetermined range in order to ensure final product quality.

Materials and methods

In order to optimize CPPs in tablet compression process and assess the impact on average mass, friability, thickness, hardness, dissolution and disintegration as CQAs, Quality by Design (QbD) approach has been applied. The goal is to approximate the response by a mathematical model for the purpose of optimization and finding a region of operability (normal operating range).

The robustness/optimization study has been performed on one industrial batch of the product in the Production Department. $2^2$ full factorial design with two replicates in the center point has been applied with varying two factors: tableting speed (tbl/hour) and main compression force (kN), adjusted to obtain low and high levels of tablet hardness on the upper and lower limit of the target range, in order to provide flexibility in the process while still producing product which meets relevant quality criteria.

Seven experiments were generated, performed and the results for each response were inserted and evaluation of raw data and model interpretation were performed using MODDE Go® statistical software.

Appropriate statistical analysis has been performed regarding the optimization/robustness results in order to estimate the influence of changes in the process parameters on the physico-chemical properties of the tablets (Eriksson et al., 2008).

Additionally, the power failure case on the compression process of the product has been tested
with process control analysis of physico-chemical parameters. The results of these experiments have been used to optimize the tablet compression process and predict the process’s ability to produce product within acceptance criteria.

**Results and discussion**

Main effects of independent variables on response variables have been evaluated. Tablet hardness increases with increase in the compression force values, due to higher compression force applied. As the compression force increases, thickness progressively decreases due to tightening of the tablet core as result of higher applied compression pressure. Tablets compressed under higher compression forces have slightly lower friability values due to higher tablet hardness. Hardness, thickness and friability are not affected from increasing tablet compression speed from low to high level. Disintegration time of tablets is not generally affected from increasing tablet compression force and tablet compression speed from low to high level. Dissolution of the API is slightly affected due to increased compression force, but the results are within predetermined limit. There is no significant change in mass variation due to change in compression forces applied and compression speed from low to high level. The variation in average mass is within in-process control limits.

Correlation plot demonstrates and confirms the main correlations of factors with the responses, therefore it can be confirmed that compression force is positively correlated to tablet hardness. Negative correlation is observed between the compression force and thickness and compression force and friability and hardness and dissolution.

From the obtained Sweet Spot Plot for the areas where the responses are within specified ranges, we can observe that all responses for concerned dependent variables hardness, thickness, friability, mass variation, disintegration and dissolution are within selected range. Varying independent variables, tablet machine speed from low level (40 000 tbl/h) to high level (60 000 tbl/h) and compression force from low level (15 kN) to high level (25 kN) provides results for concerned dependent variables within desired range.

The obtained results for all parameters for the power failure case are well within the predetermined acceptance criteria. Dissolution profile in the specification media pH 6.8 has been performed for all cases of the optimization process including for the power failure case and shows similar dissolution profiles for all cases of the tablet compression process.

**Conclusion**

Evaluation of tablet compression process by factorial design showed that the tested factors within the tested range have no significant influence on the CQAs of the final product. Each combination of the tested parameters within the tested range would produce product with acceptable quality. Robustness/optimization study of tablet compressing process has been successfully performed and defines the range of baseline compressing parameters which provide reproducibility of the results for quality attributes of the tablet cores during tablet compressing process.

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Effect of binder, lubricant and compression force levels on hardness and disintegration time of immediate release tablets with a high-dose API - a $3^3$ full-factorial design

Krume Toshev*, Natasha Anevska Stojanovska, Sonja Ugarkovic

Alkaloid AD, Aleksandar Makedonski 12, 1000 Skopje, RN Macedonia

Introduction

The skills of the formulation scientist are often put at test when a very high-dose API (>95%) with unfavorable properties is to be incorporated in an immediate release tablet. As the room for excipients in tablets with >95% API is so small, a very careful consideration should be made on the choice and concentration of each of the excipients and the parameters of the process in order to achieve desirable properties of the tablets (Lionberger, 2008).

The aim of this study is to evaluate the effect of the levels of binder, lubricant and compression force on the hardness and disintegration time of immediate release tablets with API of BCS class III present in the formulation at a very high-dose (>95%).

Materials and methods

API of BCS class III is an oral hypoglycemic agent that has very poor flow and compression properties and ability to form hard lumps with storage. The other excipients in the formulation are binder – povidone K90 and lubricant – magnesium stearate. Due to the unfavorable properties of the API the chosen manufacturing process was wet granulation.

In this study a $3^3$ full-factorial response surface design with 30 experiments (including 3 central points) is used to evaluate the effects of levels of binder – povidone K90, lubricant - magnesium stearate and compression force on hardness and disintegration time of the immediate release tablets. (Armstrong 2006). The tested levels in the formulation for the binder povidone K-30 are 3%, 4% and 5%, the tested levels for the lubricant are 1%, 1.5% and 2%, and the tested main compression force levels are 10kN, 15kN and 20kN.

For the purpose of the study, small laboratory trials of 0.5kg were made. API was obtained from manufacturer USV. API was wet granulated with the binder povidone K90 on a fluid-bed granulator after which the lubricant is added extragranularly. Each of the trials was compressed at 3 different compression forces 10kN, 15kN and 20kN on a Korsch XL 100 Pro tablet press. The disintegration time was tested on apparatus for disintegration Erweka ZT322 and the hardness was tested on a hardness tester Erweka TBH 425TD.

Evaluation of raw data and model interpretation was performed using MODDE Go® statistical software. In order to predict the variability of the results for response variables with varying the values for independent variables, Multiple linear regression (MLR) was used.
Results and discussion

The chosen model (quadratic model) was shown to be significant and fitting for the data for all responses. The predictive ability of the model is moderate to high for all responses. The observed vs. predicted response values of the responses plot shows that most results fall on the 1:1 line which indicates good model.

From the effect plots the highest effects of each of the factors binder, lubricant and the compression force were observed when the other two factors are set at medium level. The highest effect on the response hardness is from the binder povidone K90 and the compression force. The highest effect on the response disintegration time is from the lubricant magnesium stearate. We can also observe that tablet hardness increases with increasing compression force from 10–20 kN and with increasing % of binder povidone K90 in formulation from 3–5%.

Disintegration time prolongs with increasing magnesium stearate (%) from 1–2%. Disintegration time is not affected by change in compression force or binder (%). From the response surface plots the interaction of two factors can be studied when the third factor is set at medium level and their effect on the disintegration and hardness of tablets. A sharp increase in hardness can be observed when % of binder povidone K90 in formulation increases from 3–5%, the compression force is at its max 20 kN and the % of magnesium stearate is at its median level 1.5%. The same sharp increase is also observed when the compression force increases from 10–20 kN with the % of binder povidone K90 in formulation set at the max 5% and the % of magnesium stearate is at its median level 1.5% which showcases the additive effect of the compression force and the % of binder on the hardness of tablets. The lubricant magnesium stearate, on the other hand, in levels from 1% to 1.5% has very small positive effect on the hardness which turns into a negative effect when the level is increased to 2%. The disintegration time decreases sharply when the % of magnesium stearate decreases from 2% to 1%, with a very little additive effect of the compression force. It is interesting to observe that the effect of the lubricant is more pronounced on the disintegration time of the tablets than the effect of the compression force. The formulation can be further optimized to have the fastest disintegration times with the highest hardness, and also a sweet spot can be made to cover the areas where the responses are within desired ranges. From the optimization of the formulation tool in the MODDE Go statistical software, in order to achieve tablet with highest hardness and fastest disintegration time, the chosen formulation should contain 4.1% povidone K90, 1.2% magnesium stearate and should be compressed at 20 kN main compression force.

Conclusion

In order to achieve desirable properties of the tablets especially in formulations where the room for excipients is very low it is of crucial value that the excipients and their quantities are chosen wisely and the parameters of the process are set properly. The 3³ full-factorial design adequately describes the correlation of the levels of binder, lubricant and compression force and their main and interaction effects on the hardness and disintegration time of immediate release tablets with API of BCS class III.

The highest positive effects on the response hardness can be observed from the binder povidone K90 and the compression force and the highest negative effect on the disintegration time can be observed from the lubricant magnesium stearate. Additive interaction effect can be observed of the compression force and the % of binder on the hardness of tablets. The disintegration time is mostly affected by the lubricant magnesium stearate with a negligible additive effect of the compression force.

The formulation can be further optimized to achieve the fastest disintegration time with the highest hardness.

References


Effect of process scale-up on granulate, tablets properties and dissolution behavior in immediate release fixed-dose combination product

Nadica Vanova\textsuperscript{1,2,*}, Roza Markovski\textsuperscript{1,*}, Elena Kazandzievska\textsuperscript{1}, Lile Zdraveska\textsuperscript{1}, Monika Kostovska\textsuperscript{1}, Packa Antovska\textsuperscript{1}, Natasa Anevksa Stojanovska\textsuperscript{1}, Sonja Ugarkovic\textsuperscript{1}

\textsuperscript{1}Alkaloid AD, Pharmaceutical Chemical and Cosmetics Company, Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia
\textsuperscript{2}Institute of Pharmaceutical Technology, Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Teresa 47, 1000 Skopje, Republic of North Macedonia

Introduction

Fixed-dose combination immediate release (IR) tablets of a Biopharmaceutical classification system (BCS class) II antihyperlipidemic and I antihypertensive drug was developed as a generic product using wet granulation technology. Obtaining similarity to the reference product (RP) in order to assure similar release profile and further similar drug pharmacokinetics is a challenge, assuming that there are many influences on the active components dissolution behavior, where one of the main challenges is the process scale-up. Process and product understanding are needed in order to define the critical process parameters (CPP) and the critical quality attributes (CQAs). Further successful process parameters scaling and adaptation from laboratory scale to production scale is needed in order to assure the desired product quality. Different scale-up approaches and rules for wet-granulation are described in the literature, among are the constant impeller tip speed (Rekhi et al., 1996), specific power input (Campbel et al., 2011; Landin et al, 1996) or flow regime characterized by the dimensionless Froude number (Lister et al., 2002).

Even relatively minor parametric changes can have influence on the dissolution behavior, where granule strength and particle size distribution (PSD) of granules can be affected. In our study the dimensionless Froude number approach was used to scale the wet-granulation mixing speed from laboratory scale trial of 1 kg IR fixed-dose combination product from 4 L high shear mixer, to production scale trial of 32 kg produced in 65 L pilot high shear mixer by two granulation sub-batches.

The aim of the present work was to compare the granulate characteristics and dissolution behavior of active ingredients from final drug product.

Materials and methods

Formulation

BCS class II drug with low water solubility and BCS class I water-soluble APIs were wet granulated with excipients of common pharmaceutical usage with hydroxypropylcellulose dissolved in water as binder.

\* nvanova@alkaloid.com.mk; rmarkovska@alkaloid.com.mk
Production process

High-shear wet granulation and fluidized-bed drying process were used. The chopper speed and impeller speed as CPPs of the wet granulation phase were set as 1500 rpm and 150 rpm respectively, for 1 minute during the binder addition and 1500 rpm and 150 rpm respectively, for 6 minutes during the wet-massing for laboratory trial at Diosna P 4 high-shear mixer granulator. For the pilot scale 1500 rpm and 100 rpm for the chopper and impeller were set with 2 minutes for binder addition and 3 minutes for wet-massing in Aeromatic-Fielder PMA™ 65. Fluid-bed drying was carried in Hüttlin® MicroLab drier with air consumption volume of 15 m³/h and drying temperature of 60-65 °C at laboratory scale and on Aeromatic-Fielder TSG-2 using air consumption of 200-250 m³/h and drying temperature of 70-75°C. Tablets were produced using KORSCH XL 100 laboratory scale rotary tablet press machine and Fette 2090i production scale rotary tablet press machine. Tablets were film coated in O’Hara Labcoat™ laboratory perforated film-coating machine and Glatt® production scale film-coating machine of same working principle.

Testing methods

PSD was measured using vibratory sieve Retsch AS200 Control, and granule porosity was tested on AccuPyc™ gas pycnometer. The produced film-coated tablets were tested for dissolution comparability to the RP by apparatus II (paddle) method using 900 ml and 75 rpm in different pH media (2.2; 4.5 and 6.8). The dissolved % of each API was measured using in-house HPLC method. Comparison between laboratory and scale-up trial was made along with the comparison to the dissolution with the RP for both APIs.

Results and discussion

Similarity between PSD of the obtained granules on laboratory scale and pilot production scale demonstrated similarity (t-Stat<t-Critical) with obtained bulk densities for the granulate of 0.591 g/mL for laboratory trial and 0.694 g/mL for scaled-up product as well as granule porosity of 64.64%. The produced film coated tablets had similar physical characteristics. The tablet hardness which can be directly related to the disintegration and therefore to the dissolution profile of the product, was in range of 6.63-8.15 kP and disintegration time in range of 00:00:38-00:00:47 minutes for laboratory scale and 4.59-6.12 kP for the film coated tablet hardness and 00:00:45-00:00:59 for the disintegration time of the scaled-up product. As expected, the Froude number calculation for adaptation of wet-granulation impeller speed for the scale-up at a constant chopper speed and mixing time, leaded to comparable results for the granulate and tablets characteristics and comparative dissolution profiles for both BCS class II and I APIs compared to the RP and to laboratory scale. Similarity with the RP and between laboratory scale and scaled-up trial was demonstrated in each tested pH media for both APIs. Similarity $f_2$ statistic factor with mathematical calculation was used where relevant and no mathematical calculation was used where results ≥85% in 15 min were obtained, as following the conditions described in EMA guideline on the investigation of bioequivalence (CPMP/EWP/QWP/ 1401/ 98 Rev. 1/ Corr **).

Conclusion

CPPs of the granulation process were successfully scaled-up, where Froude number calculations for the wet-granulation impeller speed at constant chopper speed and mixing time were used and the comprehensive range of parameters were successfully adapted to pilot production.

References


Psoriasis therapy: Current state and future prospects

Beti Djurdjic¹,²*, Vjeroslava Slavic¹,²

¹Centre of Excellence for Biomedical Researches (CEBIMER), Institute for physical medicine, rehabilitation and rheumatology “Dr Simo Milosevic”, Sava Ilica 5, 85347 Igalo, Montenegro
²Department of Pharmacy, Faculty of Medicine, University of Montenegro, Krusevac bb, 81000 Podgorica, Montenegro

Introduction

Psoriasis is a chronic inflammatory, immune-mediated disorder that mainly affects the skin and joints. Psoriasis pathophysiology is characterized by abnormal keratinocyte proliferation and immune cell infiltration in the epidermis and dermis involving the innate and adaptive immune systems. Various types of psoriasis have been reported in literature and practice: plaque, guttate, inverse, pustular and erythrodermic psoriasis. Individuals with psoriasis are at an increased risk of developing other chronic and serious health diseases such as psoriatic arthritis, metabolic syndrome (or components of it), cardiovascular disorders, anxiety and depression, non-alcoholic fatty liver disease and Crohn’s disease (Boehncke and Schön, 2015; Greb et al., 2016).

The aim of this paper was to review the existing therapy for psoriasis, as well as utilization of nanotechnology in formulation of drug delivery systems for psoriasis therapy.

Current trends in treatment of psoriasis

Psoriasis is a chronic relapsing disease, which often require a long-term therapy. The choice of therapy is determined by disease severity, comorbidities, and access to health care (Rendon and Schäkel, 2019). Current treatments for psoriasis include topical therapies, phototherapy, conventional systemic drugs and biologic agents. Most patients with psoriasis have mild to moderate disease that can be treated safely and effectively with topical therapies, including corticosteroids, vitamin D derivates (calcipotriol, calcitriol, tacalcitol), retinoids (tazarotene) tar, keratolytic agents (urea, salicylic acid), and emollients (Brandon et al., 2019). Results from a recent meta-analysis showed that a combination of corticosteroids and vitamin D₃ was the most effective treatment for the scalp (Boehncke and Schön, 2015). Methotrexate (folic acid analogue that inhibits DNA synthesis by blocking thymidine and purine biosynthesis), cyclosporin (T cell-inhibiting immunosuppressant) and acitretin (retinoids) are systemic treatment options for psoriasis (Rendon and Schäkel, 2019). Over the past decade, several biologics have been developed and approved for the treatment of psoriasis. TNF-α inhibitors (etanercept, infliximab, adalimumab and certolizumab), first-generation biologics, are effective for plaque psoriasis and psoriatic arthritis. With the exception of etanercept, which is a fusion protein, other biologics are monoclonal antibodies. In the treatment of psoriasis, they show different PASI (Psoriasis Area Severity Index) score 75% response rates: 52%, 59%, 80% and 83% for etanercept, adalimumab, infliximab and certolizumab, respectively (Rendon and Schäkel, 2019). Currently anti–IL-17 agents (secukinumab brodalumab, ixekizumab), anti–IL-23 inhibitors (guselkumab), and anti–IL-12-23 inhibitors (ustekinumab) are commonly used agents for plaque psoriasis and psoriatic arthritis (Brandon et al., 2019). In Cochrane Review (Sbidian et al., 2020) 140 studies were collected and analysed to compare the efficacy and safety of conventional systemic
agents, small molecules, and biologics for people with moderate-to-severe psoriasis, and to provide a ranking of these treatments according to their efficacy and safety. Network meta-analysis at class level showed that all of the interventions were significantly more effective than placebo in terms of reaching PASI score 90%. At class level, in terms of reaching PASI score 90, the biologic treatments anti-IL17, anti-IL12/23, anti-IL23 and anti-TNF-α were significantly more effective than the small molecules and the conventional systemic agents. The results showed that a selection of treatments from the class of biological medicines appear to be the most effective systemic medicines for achieving a 90% improvement in PASI score.

Development of nanotechnology-based drug delivery systems for psoriasis therapy

Psoriasis therapy based on conventional formulations can provide therapeutic benefits only to a limited extent (Rahman et al., 2014). Topical therapies have limitations as treatments have the potential to cause cutaneous side effects. While skin is the desired target of topical therapies, it is also a barrier to effective drug penetration and absorption, and the keratinocyte hyperproliferation associated with psoriasis further fortifies this barrier. While oral and injectable systemic therapies overcome the need to penetrate the stratum corneum, associated infection risk as well as liver, kidney, and bone marrow toxicities can limit their use in some patients (Murphy et al., 2019). Recent advancement in nanotechnology-based drug delivery systems has led to the possibility of improving the efficacy and safety of pharmacotherapeutic agents for psoriasis. Numerous studies in recent decade has demonstrated that nanoparticles as a drug carrier (such as liposomes, transfersomes, niosomes, polymersomes, nanoemulsions, solid lipid nanoparticles, polymeric nanoparticles, nanogels) can enhance the efficacy and reduce side effects of drug agents through increased skin retention and sustained drug release. The developed nanoemulsion loaded gel for topical co-delivery of clobetasol propionate and calcipotriol showed enhanced penetration and controlled release of drugs, increased anti-psoriatic efficacy compared to free drugs (Kaur et al., 2017). Kumar et al. (2016) created cyclosporine-loaded liposomes and incorporated these carriers into a Carbopol hydrogel. The clinical efficacy was studied in double-blind, randomized controlled trial of 38 patients with psoriasis. The developed formulation resulted in higher plaque clearance than a commercial cyclosporine cream but lower clearance than clobetasol propionate.

Conclusion

The results on animal models using nanoparticles via different route seem promising, but the number of studies in humans is limited and while the short-term use of nanocarriers appears safe, long-term outcomes are unknown. While recent research has demonstrated the benefit of nanotechnology-based drug delivery systems for psoriasis, more research is needed directed toward the clinical study to establish the fate of nanoparticles and optimize drug loaded nanocarriers for clinical use (Murphy et al., 2019; Rahman et al., 2014).

References

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Formulation and evaluation of a solid self-emulsifying drug delivery system containing cefuroxime axetil

Eleonora Trajanovska¹*, Frosina Jovanovic¹, Ana Atanasova¹, Maja Hadzieva Gigovska¹, Oja Ali Memed¹, Packa Antovska¹, Sonja Ugarkovic¹, Marija Glavas Dodov²

¹Research and Development, Alkaloid AD, Blvd. Aleksandar Makedonski 12, 1000 Skopje, North Macedonia
²Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, North Macedonia

Introduction

One of the biggest problems in formulation of oral pharmaceutical dosage forms is the lipophilic nature of the drug substances, therefore, various formulation strategies have been explored in recent years such as usage of lipid solutions, emulsions and emulsion concentrates prepared as physically stable formulations suitable for encapsulation of poorly soluble drugs (Rodriguez-Aller et al., 2015). Formulation approaches, such as self-emulsifying drug delivery systems (SEDDS) have been recently used to overcome drug solubility problems (Vasconcelos et al., 2017) by using isotropic multi component system composed of oil, surfactant and co-surfactant/co-solvent, which form micro- or nano-emulsion in the presence of water.

Soft gelatine capsules are simple and commonly used dosage forms for encapsulation of liquid SEDDS, nevertheless, this technology have some limitations i.e. manufacturing and packaging process difficulties, stability of the final drug product, etc. Due to these reasons, attention has been focused on formulation of solid SEDDS in form of tablets (uncoated or film-coated tablets) (Joyce et al., 2018). In that direction, various formulation strategies have been used for solidification of SEDDS, among which the simplest approach is adsorption of the SEDDS to solid carriers–excipients, commonly used in tablet formulation (Mandić et al., 2017). The purpose of the study was formulation of a solid SEDDS containing the poorly soluble cefuroxime axetil in a form of a conventional tablet.

Materials and methods

Cefuroxime axetil (CA) was supplied from Orhid Chem. Pharm. Ltd. (India). Olive oil, talc (Parteck® Lub Talk Emprove), methanol and citric acid monohydrate were obtained from Merck KgaA, Germany. Microcrystalline cellulose (MCC-Avicel® PH 112) and croscarmellose sodium (CS-AcDiSol®) were supplied from FMC Biopolymer, USA. Polyvinylpyrrolidone (PVP-Kollidon® 30F) and Polyvinylpyrrolidone, crosslinked (PVP-C-Kollidon® CL) were supplied from BTC Chem. Distrib., Germany. Polysorbate 80 (Tween 80) was obtained from Croda Europe Lim., France, polyethylene glycol (PEG 400) from Clariant, Germany, colloidal anhydrous silica (CAS-Aerosil® 200) from Pharma Evonik Ind., Germany, magnesium stearate (MS) from FaciSpa-Carasco GE, Italy and lactose monohydrate (LM-Tablettose® 100) from Meggle, Germany.

Preparation of solid SEDDS granules and tablets

CA was successfully incorporated in the mixture of olive oil, PEG 400, Tween 80 and citric acid monohydrate (data are not presented). Incorporation of the SEDDS onto solid drug carries was done using a high shear granulation technique (all samples

* etrajanovska@alkaloid.com.mk
contained SEDDS 57% and CAS 18%; additionally, granules were prepared with: S1: MCC 13%, PVP 2.2%, PVP-CL 9%; S2: MCC 13%, PVP 2.2%, CS 9%; S3: LM 13%, PVP 2.2%, CS 9% and S4: MCC 13%, PVP-CL 9.5%; impeller speed 400 rpm, chopper speed 1000 rpm, 5 min; Diosna P1/6, Diosna Dierks & Söhne GmbH, Germany). Obtained granules (S1-4) were sieved (mesh size 18) and blended with the lubricant and glidant (Talc 0.5%, MS 0.5%; 25 rpm, 3 min; Erweka PM5, Erweka GmbH, Germany). Flow properties of the final blends were evaluated according to Ph. Eur. 9.0 (2.9.36 and 2.2.32, respectively). Prepared granules were compressed into oblong, biconvex, white tablets with length of 21 mm on rotary tablet press (Korsch Pro XL100, Germany). The tablets were characterized for: mass and uniformity of mass (Ph. Eur. 9.0 (2.9.5); Sartorius Secura 224-1 CEU, Sartorius AG, Germany), hardness, diameter and thickness (Erweka Hardness Tester 425 TD, Erweka GmbH, Germany), friability (Ph. Eur. 9.0 (2.9.7); Erweka TAR 100, Erweka GmbH, Germany) and disintegration (Ph. Eur. 9.0 (2.9.1); Erweka ZT322, Erweka GmbH, Germany). Assay analyses of CA were done using the HPLC systems (Agilent 1200 Binary Series, DAD detector, Germany and Hitachi Elite® Lachrom, Hitachi, USA), column (Inertsil® ODS-2, 4.6x150 mm, 5µm, GL Science, Japan). As a mobile phase, 20% methanol and 80% water purified were used, with chromatographic conditions: mobile phase flow of 1 mL/min, column temperature of 40 °C, injection volume of 100 µL at 281 nm. In vitro CA release from the tablets (n = 3) was carried out with USP Apparatus II using 900 mL, 0.07 N HCl as dissolution media (Varian Vankel 7025 Model 115/230, Varian, USA) at 37±0.5 °C and 100 rpm. At predetermined time intervals (after 5, 10, 15, 20, 30 and 45 min.), 10 mL were withdrawn and filtered (0.20 µm) and were and analyzed by above mentioned HPLC method.

Results and discussion

Prepared granules showed fair (S1-2) to good flow (S3-4) properties, considering the high oil loading in the formulations. Characterization of the physical properties of the tablets showed that all obtained results are within the predetermined acceptance criteria (tablets mass of 1100 mg ± 5% with 21±0.3 and 6.5±0.2 mm for tablet diameter and thickness, respectively, 2-5 kP hardness, friability of max 1% and disintegration time of max. 15 min.

Determined CA content and the drug release studies from different tablets as solid SEDDS formulations showed that sample S3 released ~ 87% of CA during the period of 15 min. and more that 96% after 45 min. (99.6% CA content), thus complying with the USP specification for conventional CA tablets. In comparison, the in vitro release of CA (125 mg) as a powder was 8 and 13%, respectively, in the same time intervals and under the same test conditions.

Conclusion

Conventional immediate release tablets containing SEDDS of CA were prepared using simple solidification with adsorption of the SEDDS onto solid carrier containing different excipients. Based on in vitro dissolution studies, selected sample will be further evaluated for formulation optimization.

References

Compounded omeprazole suspension - stable or not?

Ognjenka Rahić*, Edina Vranić, Jasmina Hadžiabdić, Merima Sirbubalo, Amina Tucak

Faculty of Pharmacy, University of Sarajevo, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina

Introduction

Omeprazole is a proton pump inhibitor commonly used in pediatric patients (Wensel, 2009). Pediatric patients are usually unable to swallow solid dosage forms and they need dose adjustment. Therefore, the dosage form of choice for this population is compounded liquid preparation.

Since pharmacies don't usually dispose of pure active substances, compounded liquid preparations are most commonly prepared from commercially available solid dosage forms, in a way that tablets are simply pulverized or capsule contents emptied, adding water or one of the commercially available vehicles (Haywood and Glass, 2013).

Considering the risks associated with the preparation and use of compounded preparations, the Chapter <795> of the US Pharmacopoeia states that the beyond-use date is 14 days for non-preserved aqueous oral formulations, if stored in the refrigerator. Preserved aqueous preparations can be stored for 35 days at controlled room temperature or in the refrigerator (USP, 2015).

Materials and methods

A review of the reference articles on the stability testing of compounded omeprazole suspensions has been conducted. Only articles with stability studies using stability indicating methods have been considered.

Results and discussion

Omeprazole suspension was prepared from pellets emptied from capsules with the addition of different excipients. Formulation 1 (F1) contained glycerol, carmellose sodium, simple syrup, and a combination of methyl and propyl paraben. The vehicle in the specified formulation was purified water. F1 was highly unstable (Milic et al., 2017). This is not a surprise, since the formulation does not contain agents to increase the pH value, which is necessary, given that the degradation of omeprazole occurs at the pH below 7.8. Given the stated, it is imperative to develop a formulation whose pH is above 8.

Formulation 2 (F2) contained 8% w/v sodium bicarbonate, methyl and propyl paraben and propylene glycol. Vehicle was purified water. Sodium bicarbonate is included in F2. It maintains high pH while acting as a suspension agent (Whaley et al., 2012).

Formulation 3 (F3) was like F2, with the addition of xanthan gum. Xanthan gum, as a viscosity increasing agent, should increase the physical stability of the suspension. However, the modification of the rheological characteristics in this case did not lead to an improvement in the stability. F2 and F3 have proved to be stable 30 days in the refrigerator (Milic et al., 2017).

Formulation 4 (F4) was prepared by dispersing omeprazole pellets in 8.4% w/v sodium bicarbonate solution. This formulation has been shown to be stable for up to 14 days.
days at room temperature and up to 30 days in the refrigerator. In F4 the optimal pH is provided, due to the presence of sodium bicarbonate. Although no preservatives were added, microbial count was within the limits (Quercia et al., 1997).

Formulation 5 (F5) of omeprazole suspension is also made in 8.4% w/v sodium bicarbonate solution, but by dispersing pure omeprazole. The shelf life for this suspension was 45 days, if stored in the refrigerator (Allen, 2018). The fact that F5 was prepared with omeprazole pure substance, presumably resulted in a longer shelf life.

Formulation 6 (F6) of omeprazole suspension was prepared from pure omeprazole, which was subsequently mixed with SyrSpend SF Alka powder, and dispersed in purified water. F6 is stable for 92 days, if stored in the refrigerator. SyrSpend Alka powder consists of modified starch, calcium carbonate and sucralose. Modified starch acts as a suspending agent, calcium carbonate provides a pH above 8. In addition, due to the unpleasant, bitter taste of omeprazole, which sodium bicarbonate in previous formulations further intensifies, the formulation of SyrSpend Alka included sweetener, which masks the unpleasant taste of the active substance (Whaley et al., 2012).

Formulation 7 (F7) of omeprazole suspension was obtained by reconstitution of omeprazole FIRST Compounding Kit. This kit consists of pure omeprazole and vehicle containing: strawberry aroma, benzyl alcohol, color, sweetener, poloxamer, propylene glycol, water, simethicone emulsion, sodium bicarbonate, sodium citrate, sucralose and xanthan gum. Beyond-use date for this suspension is 30 days, if kept in the refrigerator (Allen, 2019). Although, F7 contained more suspending agents, preservatives and buffers, it was not more stable than F6.

**Conclusion**

Stable omeprazole suspension is obtained by selecting the appropriate preparation process as well as the appropriate formulation. Suspensions prepared from pure omeprazole proved to be more stable than the ones prepared from omeprazole pellets.

The most stable suspension was F6 with the proven beyond-use date of 90 days in the refrigerator. The vehicle used consists of only three substances, which is a significant advantage over the others, because there is less possibility of interactions. It provides a pH above 8, which is an absolute imperative from the aspect of the stability omeprazole. In addition, it contains sweetener, which improves the compliance of young patients. Additional advantage of this vehicle is that it does not contain sorbitol, ethanol, as well as propylene glycol and other preservatives. That makes it very suitable for newborns. When added simplicity of handling and quick preparation of this suspension in pharmacies, F6 can be considered as a formulation of choice for pediatric patients.

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Importance of stabilizers of nanocrystals of poorly soluble drugs

Jasmina Hadžiabdić*, Semra Brekalo, Ognjenka Rahić, Amina Tucak, 
Merima Sirbubalo, Edina Vranić

1Faculty of Pharmacy, University of Sarajevo, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina

Introduction

Approximately 70-90% of the new active pharmaceutical ingredients/drugs are poorly soluble in water/biological fluids. Improvement of solubility, dissolution rate, bioavailability are the main characteristics of drug nanocrystals that are important for oral drug administration. High bioadhesive activity, depending on the type of stabilizer, is considered to be an essential feature of drug nanocrystals for oral, dermal, ocular dosage forms (Chang et al., 2015; Sheokand et al., 2018; Tuomela et al., 2016). Drug nanocrystals are solid nanosized particles of pharmacologically active substances, mainly BCS class IIa and IIb, 200 to 600 nm in diameter, homogeneously coated with 10-50% stabilizer/surfactants and/or polymers, forming ultrafine dispersion (Malamatari et al., 2018). Drug nanocrystals are usually in the crystalline state, but depending on the manufacturing method and process parameters, they may be in the amorphous state (Shete et al., 2014). Drug nanocrystals can be obtained by increasing their particle size by controlled precipitation/agglomeration from solution or by reducing drug particle size by milling to the desirable size. The two basic methods for obtaining drug nanocrystals are bottom up (e.g., precipitation) and top down (e.g., milling) methods, or drug nanocrystals can be made by a combination of these processes. By combining these two methods the desired particle size of drugs can be achieved and disadvantages of the individual methods are overcome. These methods are intended for the preparation of liquid pharmaceutical nanosuspensions whose internal phase consists of drug nanocrystals particles, which can be converted into solid drug nanocrystals by post-production processes (spray drying, freeze drying or other process) in order to improve chemical, physical stability of drug during storage, when the selected stabilizer of drug nanocrystal could not provide long-term stability of the liquid nanosuspension (Sheokand et al., 2018).

Classification and role of stabilizers of drug nanocrystals

In order to obtain physically stable drug nanocrystals ionic surfactants, non-ionic surfactants, synthetic linear polymers, synthetic co-polymeric, semi-synthetic ionic polymers, semisynthetic non-ionic polymers, food proteins, amino acids and co-polymers are used as stabilizers (Chang et al., 2015; Shete et al., 2014). Stabilizers provide electrostatic (electrostatic repulsion) (ionic polymers/surfactants), steric (steric hindrance) (non-ionic polymers/surfactants) or electrosteric (mixture of ionic and non-ionic polymers/surfactants) stabilization of drug nanocrystals (Chang et al., 2015; Peltonen and Strachan, 2015; Sheokand et al.,

* jasmina.hadziabdic@ffsa.unsa.ba
Stabilizers provide physical stability of drug nanocrystals by reducing their free surface energy, reducing their hydrophobicity, inhibiting the aggregation of their particles, Ostwald ripening, changing the crystalline shape of nanosized particles during preparation and storage (Chang et al., 2015).

Selection of stabilizers of drug nanocrystals

The selection of stabilizer depends on the properties of the active substance and the stabilizer, affinity and interaction between them, concentration of the drug and the stabilizer, the method of manufacture, the route of administration, the pH of the medium to which it is exposed. Drug nanocrystals who have minimal size differences are produced by the addition of a polymer that has surface energy similar to the drug. A combination of a drug and a stabilizer with a similar log P value (lipophilicity), having approximately equal hydrophobicity, form stable nanosuspensions. Drugs with high enthalpy of melting are preferred (Shete et al., 2014). Polymer stabilizers such as hydroxypropylcellulose and hydroxypropyl methylcellulose, polyvinylpyrrolidone K30 and Pluronic® (F68 and F127) with higher molar mass (50 kDa-100 kDa) are more effective steric stabilizers than those of lower molar masses (Malamatari et al., 2018). The affinity of the stabilizers for the drug particle surface influences absorption rate of the drug (Shete et al., 2014). The stabilizer concentration is usually low, but must be high enough to completely cover the surface of the drug nanocrystals and result in physical stability of drug. Higher stabilizer concentration leads to Ostwald ripening, while insufficient stabilizer concentration leads to the aggregation of the drug nanocrystals. Due to the low stabilizer concentration, drug nanocrystals are safe for parenteral administration (Chang et al., 2015; Malamatari et al., 2018; Shete et al., 2014).

Conclusion

The role of stabilizers is to physically stabilize drug nanocrystals. Ionic surfactants (e.g., sodium lauryl sulfate) below a critical micellar concentration have much better stabilization potential than non-ionic surfactants (e.g., Tween 80) because they provide surface charge to drug particles that provides their electrostatic repulsion. Ion stabilizers are very sensitive to changes in pH value. The length of the polymer chain should be of sufficient size to overcome the attraction Van der Waals forces between drug particles, to prevent their aggregation due to short or too long polymer chains (chain collapse). Most stabilizers bind for nanosized particles of pharmacologically active substances by hydrophilic-hydrophobic interactions and increase their wetting. The most commonly used steric stabilizers are: hydroxypropyl methylcellulose, hydroxypropylcellulose, polyvinylpyrrolidone K 30, Pluronic® F68, Pluronic® F127, D-α-tocopherol polyethylene glycol 1000 succinate, electrostatic stabilizers are: sodium lauryl sulphate, sodium docusate, and of electrostatic stabilizers: sodium carboxymethylcellulose. More efficient physical stability of drug nanocrystals is achieved by a combination of stabilizers (surfactants and polymers: sodium lauryl sulfate and hydroxypropyl methylcellulose, Pluronic® F68 and lecithin).

References

Fabrication of 3D-printed PLA microneedles as physical permeation enhancers in transdermal delivery

Merima Sirbubalo¹, Mirela Camović¹, Amina Tucak¹, Kenan Muhammedagić², Ognjenka Rahić¹, Jasmina Hadžiabdić¹, Lamija Hindija¹, Ahmet Čekić², Marija Glavas-Dodov³, Edina Vranić¹*

¹Faculty of Pharmacy, University of Sarajevo, Zmaja od Bosne 8, 71000 Sarajevo, B&H
²Faculty of Mechanical Engineering, University of Sarajevo, Vilsonovo šetalište 7a, 71000 Sarajevo, B&H
³Faculty of Pharmacy, University Ss. Cyril and Methodius, Majka Tereza 47, 1000 Skopje, N.Macedonia

Introduction

Many different and innovative approaches have been investigated to reduce the barrier effects of the stratum corneum (SC) and one of those are microneedles. Microneedles (MNs) are micron-sized needles which assist drug delivery through skin by creating microchannels (micron-scale pores) in SC that are large enough to enable drugs, including macromolecules, to enter the skin while being small enough to avoid pain, irritation and needle phobia. They have the capacity to play a role in modern healthcare as they reduce pain, tissue damage and transmission of infection and have potential for self-administration in comparison to traditional needles. MNs have been fabricated by a variety of methods, from a range of materials (including silicon, glass, metal, carbohydrates and polymers) and in varying geometries (Quinn et al., 2014).

Additive manufacturing (AM), more commonly known as three-dimensional (3D) printing represents a new, cutting-edge technology of 3D objects fabricated from a digital model generated using computer-aided design (CAD) software by fusing or depositing proper material (e.g., ceramics, liquids, metal, plastic, powders or even living cells) in layers. Suitable thermoplastic material in the form of a filament is fed into the printer by rollers, where it is heated to just above its softening point (glass transition temperature, Tg) by heating elements into a molten state. The melted or softened material guided by gears is moved towards heat end where it is extruded from the printer’s head, through a nozzle and subsequently deposited layer-by-layer on a build plate, cooling and solidifying in under a second. The printer’s head moves within the x- and y-axes, whereas the platform can move within the z-axis, thus creating 3D structures (Alhnan et al., 2016; Goole and Amighi, 2016; Jamróz, 2018; Prased and Smyth, 2016).

The aim of this work was to fabricate biodegradable PLA microneedles using innovative FDM 3D-printing technology on two different 3D printers and then chemically etch their arrays to obtain ideally sized and shaped needles.

Materials and methods

Materials

PLA filaments were purchased from 3D Republika, Serbia. KOH and NaOH were ordered from Sigma Aldrich, Germany.

Fabrication of 3D-printed PLA microneedles

* merima.sirbubalo@ffsa.unsa.ba
Microneedles with different heights (0.6 mm, 1.2 mm and 1.8 mm) and different number and orientation of single arrays on the base (5x5, 3x3, 1x5) were designed using Ultimaker Cura software and printed using two printers with different technical specifications (Ultimaker S3 and Ultimaker 5S 3D printer, Ultimaker, Netherlands).

The following print parameters were set up: print speed 15 mm/s at 190 °C, infill density was 100%, built plate temperature was 60 °C, microneedle diameter 0.5 mm, and microneedle base diameter 1 cm. PLA filament (2.85 mm) was used as the printing material.

**Chemical etching and physical stability**

Microneedles were etched using the wet etching process in a NaOH or KOH solution (Sigma Aldrich) with different concentrations (1, 3, 5, 9 M). Microneedles were divided in two groups. First group was placed in prepared KOH solutions for 4 h, such that only the peaks were sunk and then were washed several times with distilled water. After 4 h, microneedles were completely submerged in solutions for additional 5 h (Luzuriaga et al., 2018).

On the other hand, in second group microneedles were completely submerged in NaOH solutions for 6h. After etching, microneedles were finallay washed and observed by optical microscope (MejiML2000).

Physical stability was tested using hardness apparatus TB24 (Erweka, Germany).

**Results and discussion**

The physical stability of the microneedles was significantly higher when printing with the Ultimaker S5, compared to the Ultimaker S3. Also, a resolution of printing sizes below 0.5 mm (0.25 mm), and printing different shapes of microneedles, is not possible with the Ultimaker S3 printer. Nevertheless, Ultimaker S3 was able to print multiple models of microneedles at once (6 copies of microneedles at a time) without compromising the precision of single-needle while Ultimaker S5 shows significantly less precision when printing multiple copies at a time. Due to the printer resolution, the best results are achieved by printing microneedles with higher height (1.8 mm). 5x5 orientation of single arrays on the base resulted in more accurately printed microneedles without a lot of waste material between the needles.

The etching process, in both groups reduces the thickness of the microneedles, and the best results were achieved with 5 M and 9 M NaOH or KOH over a time of 9 hours. 1 M and 3 M solutions of NaOH and KOH did not show satisfactory removal of the waste material between the needles.

All microneedles printed on Ultimaker 5S have withstood a pressure of over 150 N without breaking.

**Conclusion**

Best results were obtained with Ultimaker 5S. Printing parameters can easily be adjusted to develop microneedles of optimal height and orientation of single arrays on the base. We envision using solid microneedles in combination with current transdermal patch technology. Integrated into a patch, microneedles may provide a minimally invasive method to increase skin permeability for diffusion-based transport that could make transdermal delivery of many drugs possible, including large molecules such as proteins.

**References**


Characterization of physicochemical properties of substances using chromatographic separation methods

Natalija Nakov*, Jelena Acevska, Katerina Brezovska, Zoran Kavrakovski, Rumenka Petkovska, Aneta Dimitrovska

Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, R. North Macedonia

Introduction

Convenient and high throughput methods for characterization of physicochemical properties of substances are highly demanded in modern pharmaceutical research and industry. It is well recognized that the lipophilicity (logP) is correlated to the pharmacokinetic properties of the active pharmaceutical ingredient (API), making this physicochemical property essential for drug candidate screening. The dissociation constant (pKa) is one of the key properties during the drug formulation stage, since pH-solubility, lipophilicity and salt formation are pKa dependent. An accurate estimation of the pKa value for the API and its degradation products/impurities is also important for the analytical method development, providing ruggedness and faster method optimization (Kerns & Di, 2004).

Numerous computational and experimental methods have been exploited for determination of logP and pKa value. The shake-flask method as a gold standard for assessing logP, and potentiometry and spectrophotometry as traditional methods for pKa determination, are time consuming, sensitive to impurities and require larger quantity of pure substance having good water solubility (Reijenga et al., 2013).

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) emerge as a method of choice for characterization of pKa and logP due to several reasons: require small amount of sample, capacity to deal with impure and complex samples, and good level of throughput.

pKa assessment based on RP-HPLC

The RP-HPLC determination of pKa is based on different chromatographic retention time (Rt) of the neutral and ionic form of the substance. The isocratic RP-HPLC approach includes series of isocratic experiments at different pH value of the mobile phase, using at least three different concentration of the organic modifier (Manderscheid & Eichinger, 2003). Several years ago, pH gradient mode was developed to speed up the throughput. This approach was proposed for rapid determination of pKa value in complex mixtures (Wiczling et al., 2006).

The apparent pKa value could be determined from the plot retention factor (k’) versus pH value at a particular organic content concentration using nonlinear regression analysis software (Nakov et al., 2020). The aqueous pKa (at “zero organic solvent”) can be estimated using several mathematical models such as the Yasuda-Shedlovsky linear extrapolation method (Wiczling et al., 2006), or different empirical equations (Angelov et al., 2008; Kazakevich & Lobrutto, 2007). Evasion of the complex aqueous pKa calculation could be obtained...
using the solely aqueous RP-HPLC approach (Volna et al., 2017).

**Lipophilicity assessment based on RP-HPLC**

According to solvophobic theory, the \( R_t \) in RP-HPLC is governed by lipophilicity, meaning that the \( R_t \) is directly related to the compound’s dynamic distribution between the stationary and mobile phase. The lipophilic index is derived from the \( R_t \) that is converted to the \( \log k' \). Isocratic \( k' \) represents a relative scale of lipophilicity. However, the extrapolated \( \log k'_w \) (at “pure water”) is considered as more representative lipophilic indexes. The \( \log k' \) and \( \log k'_w \) values could be directly correlated to octanol-water \( \log P/\log D \) via Collander equation (Liang & Lian, 2015).

In isocratic approach for \( \log P \) determination, at least four isocratic mobile-phase rations are necessary to ensure reliability for linear fitting between the \( \log k' \) and organic modifier content. The gradient elution approach speeds up the experimental work, but the mathematical calculation are far more complex (Wiczling et al., 2006, Valko, 2016). The gradient \( R_t \)s are measured and converted to Chromatographic Hydrophobicity Index (CHI) values. The CHI value is the percentage of organic modifier required to achieve equal distribution of the analyte between the mobile and stationary phase. The CHI value is well correlated to \( \log P \), allowing estimation of \( \log P \) from a single gradient experiment.

In recent years, the use of Immobilized Artificial Membrane (IAM), provide an alternative way to measure lipophilicity and cell membrane permeation (Giaginis & Tsantili-Kakoulidou, 2008). The IAM columns permit the use of solely aqueous mobile phase, leading to directly measurement of \( \log k'_w \).

**Concluding remarks**

The greater use of the chromatographic principles for the characterization of physicochemical properties demonstrates their utility and predictivity, making these methodologies well recognized by the pharmaceutical industry. The future perspective is directed towards development of more reliable theoretical approaches and high throughput LC methods for characterization of physicochemical properties of substances using biomimetic stationary phases.

**References**


Quality study of insulin formulations

Blerta Pajaziti¹, Attila Gaspar², Melinda Andrási², Dashnor Nebija³, Rumenka Petkovska¹*

¹Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, North Macedonia,
²Department of Inorganic and Analytical Chemistry, University of Debrecen, Egyetem tér. 1, 4032 Debrecen, Hungary
³Department of Pharmacy, University of Prishtina “Hasan Prishtina, Bulevardi i Dëshmorëve, p.n., 10000 Prishtina, Kosovo

Introduction

Diabetes mellitus is one of the most common metabolic diseases in the world. It is caused by an absolute or relative lack of insulin activity, leading to hyperglycemia. The most common treatment of hyperglycemia caused by Type 1 and 2 Diabetes mellitus is human insulin produced by recombinant DNA techniques which is structurally identical to endogenously produced human insulin. (Waller et al., 2005). In addition, a number of insulin analogs produced by modifying the structure of human insulin with the aim to change the rates of absorption and duration and time to action are available.

Currently two type of insulin analogues are available, rapid acting analogs (insulin lispro, insulin aspart and insulin glulisin) and long acting analogs: (insulin detemir, insulin glargine and insulin degludec) (www.ema.europa.eu). Despite conventional vial and syringes methods, insulin formulations currently are administered using different prefilled insulin pen devices and also continuously using an insulin pump (Peters et al., 2016).

Orthogonal analytical methodologies including spectroscopy, chromatography, thermal analysis, electrophoresis, immunoassays, and bioassays are required to completely characterize biological medicines and examine their degradation profiles (Banga, 2006).

According European Pharmacopoeia, HPLC and peptide mapping are used for the evaluation of identity of human insulin and its analogs, whereas size exclusion chromatography (SEC) is used to test impurities with molecular masses greater than insulin/insulin analogs. In addition, RP-HPLC is used for the assessment of related substances and for the quantitative determination of active ingredient (European Pharmacopoeia, 2020).

Capillary electrophoresis (CE) has been established as a suitable technique for the quality study of biological drugs. This technique offers numerous advantages, including simplicity, high speed, excellent resolving power, sensitivity, low sample size requirements, low solvent consumption and ease of automation (Voeten et al., 2018).

The principal aim of this study was to develop a simple and fast CZE (capillary zone electrophoresis) /CGE (capillary gel electrophoresis) technique for the quality study of recombinant human insulin and its analogs, in regard to identification and the assessment of their purity and structural integrity.
Materials and methods

CZE method

For CZE separations between each injection, the capillary was preconditioned for 5 min with the running buffer and post conditioned with 70 mM SDS for 3 min, 1 M NaOH for 5 min and the buffer electrolyte for 4 min to remove all possible adsorbed materials. The buffer used was 50mM ammonium acetate pH 9. The samples were introduced at the cathodic end of the capillary; injections were performed using -50 mbar pressure for 4 s.

CGE method

For CGE separations the precondition procedure was 1 M NaOH for 10 min, 1 M HCl for 7 min and the sieving matrix for 25 min. The buffer used was a commercially available sieving matrix gel buffer (SDS-MW) by Beckman Coulter. The samples introduced using +7.5 kV for 50 s.

Results and discussion

Experimental data showed that CZE was able to separate charge variants of insulin and its analogues. Capillary electrophoresis method resulted in the efficient separation of insulin/insulin analogs and their main impurities in less than 2 minutes, which is the obvious advantage in comparison to RP-HPLC method which currently is an official pharmacopoeia method.

CGE was used to separate the higher molecular weight transformation products. These products are unable to be separated by CZE, because in the case of dimer and other polymeric complexes of insulin, the m/z ratio is still the same as the monomer of insulin. CGE is able to separate components depending on their mass.

Conclusion

In the present study the suitability of CZE and CGE method could be successfully demonstrated for the assessment of quality of pharmaceutical formulations containing human insulin and its analogs.

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References


RP-HPLC a valuable tool in monitoring dissolution test of fixed combination dosage forms

Ela Hoti*, Fabiola Noga, Aurora Tafa, Elton Myftari

Department of Pharmacy, Faculty of Medicine, UMT, Rruga Dibres 371, 1001 Tirane, Albania

Introduction

Fixed dosage forms usually contain two or more active ingredients which are responsible for a combined therapeutic activity. The combination of three well established antihypertensive agents: amlodipine, valsartan and hydrochlorothiazide in a dosage form, has been found successful for the treatment of moderate to severe hypertension (Deeks, 2009). Many analytical methods have been reported for simultaneous estimation of the active ingredients in this triple combination (Sharma et al., 2014) with HPLC being a method of choice. Different researchers have worked at applying developed and validated RP-HPLC methods in monitoring dissolution test of tablet formulations containing two or more active ingredients in combination (Celebier et al., 2010).

The aim of this work was finding a suitable HPLC method that can be used to monitor the dissolution test of two different solid dosage forms in our laboratory.

Materials and methods

Chemicals and reagent

Two commercially available tablet formulation containing 5mg amlodipine (AML), 160 mg valsartan (VAL) and 12.5 mg hydrochlorothiazide (HCT) (formulation A and formulation B) were used in this study. Working standard substances were kindly donated by TrePharm Pharmaceutical Co., Prishtina, Kosovo. Potassium dihydrogen phosphate, orthophosphoric acid, monobasic potassium phosphate, sodium hydroxide, phosphoric acid and acetonitril (HPLC grade) were purchased from Krijon sh.p.k Tirana, Albania. Distilled water was obtained by using GFL Double Water Distillation 2102 and used within 4 hours.

Instrumentation and analytical method

Analysis were performed by a HPLC (Varian Prostar, equipped with a binary pump, manual injection, volume of the loop 20 μL); on a Hipersil C18 ODS column (250 × 4.6), 5μm particle size. The isocratic mobile phase consisted of acetonitrile and potassium dihydrogen phosphate buffer (pH 3, 0.05 M) in the ratio of 40:60 v/v. The mobile phase was filtered through 0.45 μm membrane filter PTFE, degassed in an ultrasonic bath and pumped from the respective solvent reservoir to the column at a flow rate of 2 mL/min. All analyses were carried out at 25 °C and the detection wavelength set at 227 nm. The injection volume was 20 μL.

Dissolution tests conditions

The dissolution test was performed in compliance with United States Pharmacopoeia (USP) (711) using apparatus 2 (Varian 705 DS) with paddles (USP, 2017). The medium selected was phosphate buffer of pH 6.8. Paddle speed was settled at 50 rpm as stated in product monograph. Media volume of 900 mL was filled in six baskets and two baskets were used as blank for replenishing. The medium, before processing, was degassed via sonication.
process, and temperature was set at 37±0.5 °C. Samples (n=6) were drawn off at different time intervals (0–30 min).

**Method Validation**

The RP-HPLC method was validated for linearity, selectivity, accuracy, precision as per ICH guidelines (ICH, 2005). Four working standard concentrations were prepared from stock solution. The calibration curves were developed by plotting peak area versus concentration (n=5) for each of the active ingredients. The linearity of peak area responses concentrations was demonstrated by linear regression analysis.

**Results and discussions**

A chromatographic method reported in literature (El-Gizawy et al., 2014) for simultaneous estimation of the three active ingredients in a dosage form was chosen to monitor the dissolution test of two commercially available tablet formulations. The method was optimized: in terms of the flow rate used and column conditions adapted to suit our instrumentation, i.e. isocratic elution at 2 mL/min instead of 0.8 mL/min. Also, the mobile phase pH was adjusted to pH 3 instead of the reported pH value. The optimization of RP-HPLC method resulted in lower retention time of active ingredients. Regression analysis showed that the correlation coefficients for AML, VAL and HCT were found to be 0.998< $R^2$<1 and linear equations $y=16.01+23.885$ (AML), $y=33.095x-452.68$ (VAL), $y=60.534x-80.657$ (HCT).

The HPLC chromatograms obtained prove the selectivity of the method. The peaks of AML, VAL and HCT were confirmed by comparing retention time values of sample with those of standard solutions. The RSD values less than 2% for all active ingredients indicate the precision of the method. The chosen RP-HPLC method proved a high degree of accuracy, linearity, selectivity and precision.

The dissolution test of two tablet formulations containing AML, VAL and HCT was evaluated in 37±0.5 °C for 30 minutes. At different time intervals [0, 5, 10, 20, and 30 min (n=6, samples were drawn off at each time interval)], the release rate of tablet dosage form having AML, VAL and HCT was noted. The claimed dissolution and experimental results have adequate similarity. Both formulations fully comply with the pharmacopeia requirements. In 30 minutes 75% of amlodipine, 80% of hydrochlorothiazide and 80% of valsartan were released from both formulations.

**Conclusion**

The dissolution test and calculated profiles for each ingredient in formulations A and B can be considered satisfactory. The optimized RP-HPLC can be applied for simultaneous quantitative evaluation of amlodipine, valsartan and hydrochlorothiazide combined in a dosage form and in monitoring the dissolution test. The isocratic method is simple and has an affordable cost.

**References**


Competency-based training system

Mena Ivanoska Zdravkovska*, Silvija Saveska, Dafinka Damcevska, Blagica Samarova Stoey, Tatjana Bogovska, Nada Stojanoska, Milena Dobrjkovic Shotaroska, Marina Mandzukovska Micevska, Hristina Babunovska

Alkaloid AD-Skopje, Pharmaceutical, Chemical and Cosmetics Industry, blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of Macedonia

Introduction

For years many companies electronically tracked employee training and provided exams to determine that an employee was qualified to do the assigned job. A new requirement for the organizations is to establish a process for assessing existing staff competencies under its control.

Regulatory background

A review of the most widely reference ISO standards reveal the need for competent staff and assessment of their competence. All clauses requires organizations shall determine the necessary competence, provide training or take actions to satisfy competency gaps, evaluate the effectiveness of the actions taken, maintain records of education, training, skills and experience and retain appropriate documentation as evidence of competence.

Competency-Based Training Programs

The competency-based programs will vary greatly and are unique to the product being manufactured or services provided. The type of product manufactured or services provided, and the level of education, training, and experience of the personnel determine the elements of a competency-based training program. To establish and maintain a competency-based training program, the following steps must be taken:
1. Identify competency-based training requirements and needs;
2. Deliver training content;
3. Evaluate the effectiveness of training.

Training is provided for many reasons. Typically, a company performs training due to improving employee skills, compliance requirements, safety issues, changes to processes, quality improvements, and so on. Training alone is not enough to demonstrate competence; this must be demonstrated through tests, observations, and assessments. Should be provided objective evidence in order to determine that the competency requirements have been met. The questions that need to be answered are the following:
• Are records maintained and periodically reviewed?
• How does your organization determine the necessary skills and competence of person(s) doing work under its control that affect its QMS?
• Are training matrixes periodically reviewed for updates? Is this documented?
• What type of assessments do you use to evaluate competence?
• How and when do you perform assessments?
• What actions are taken when training is deemed ineffective?

* mivanoskal@alkaloid.com.mk
• What actions do you take when an employee is not competent to perform the tasks assigned?

Identify competency-based training requirements and needs

Organizations should establish a process for assessing existing staff competencies against changing business needs and prevailing trends. The first step is to identify which competencies are needed in line with the stated job functions. These competencies should be supported by training, education, and experience. To expand on this, the organizational matrix must be built for each position which is tied back to the approved job description. Starting with the job description the organization should identify the education level, type of education, and experience. Documented proof that the individual has the education and experience must be captured and available. The next step is to build a matrix comparing a specific job to the actual training requirements and the competence level required of each training requirement. The types of training should include:
• New employee orientation (company and department)
• Department-specific training
• Job-specific technical training
• Work-area-specific training (safety, SOP, etc.)
• Industry-specific ISO and regulatory training
• Supervisory training

Evaluate the effectiveness of training

What is important is the evaluation of training effectiveness and ongoing evaluation of how competent the employees are performing their duties. By performing such evaluations, the organization will be able to determine if additional training is required to achieve competency, the quality of the training material, or if staff should be reassigned due to inability to achieve competency.

The following are standard ways to assess training:
• Take a test at the beginning and end of training
• Evaluate in a real time situation with a subject matter expert (SME)
• Prepare self-assessment
• Feedback from the team or coworkers
• Manager assessment

• Customer assessment

The above process will give you what is known as a 360-degree assessment. This will yield the most accurate results because it rates the individual as objectively as possible from every angle. Once all assessments have been completed, the average of the scores are taken to find the competence score. After the assessments have been completed, the individual’s competence is determined, gaps identified, and a new training path determined.

In summary

Once an organization has identified all the training requirements and the level of competency for each requirement, these requirements and competency levels are assigned to individual employees. These requirements and competencies are compared against the training and competency each employee has completed. The gap between required training competence and completed training defines what additional training is required of the employee. Training alone does not determine competence. The effectiveness of the training must be evaluated and the competence of the individuals through skill checks and evaluations.

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On-going stability studies in climatic zones III and IV

Milena Nanov*, Nadica Boeva, Kristina Jozikj, Ana Vavlukis, Jana Pop Nikolova, Stefan Davidovski, Sanja Despotovska

Alkaloid AD-Skopje, Pharmaceutical, Chemical and Cosmetics Industry, blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia

Introduction

The climate is different in all countries in the world. Stability studies of the drug product and drug substances should be done according to the climatic conditions of the country. Stability testing is a routine procedure that is performed to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of variety of parameters, and to establish a re-test period for the drug substance or a shelf life for the drug product and recommended storage conditions.

Any physical, chemical, microbiological or change in container closure system in the product, potentially impact the efficiency and integrity of the final product and may therefore directly or indirectly impact patient`s health.

Materials and methods

There are several regulations and guidelines for stability studies of drug products and drug substances. According to the World Health Organization for stability testing of drug product and drug substances, the climate of the world is derived into five different zones: Zone I: temperate; Zone II: Mediterranean/subtropical; Zone III: hot and dry; Zone IVa: hot humid/tropical and Zone IVb: hot/higher humidity. At a WHO meeting entitled “Stability Studies in a Global Environment”, held in Geneva, December 2004, where discussions regarding stability testing for registration in climatic zone III or IV caused confusion and uncertainties, were adopted changes to stability testing requirements at an international level resulting in the following stability long-term study conditions for hot and humid climates (WHO Technical Report Series, no. 1010, Annex 10):

- 30°C/65% RH e.g. WHO, ICH, SADC, GCC, Brazil
- 30°C/70% RH e.g. EHO previous, Cuba, Brazil previous
- 30°C/75% RH e.g. ASEAN

These changes were based on new calculations and discussions where some countries in climatic zone IV expressed their wish to include a larger safety margin for medicinal products to be marketed in their region than previously foreseen in ICH Q11F (Stability Data Package for Registration Applications in Climatic Zone III and IV) (ICH guideline Q1A (R2)).

Results and discussion

As a consequence, several countries and regions have revised their own stability testing guidelines, defining up to 30°C/75% as the long-term storage conditions for hot and humid regions. The ICH Steering Committee has decided to withdraw ICH Q1F and to leave the definition of storage conditions
in Climatic Zones III and IV to the respective regions and the WHO. In assessing the impact of the withdrawal of ICH Q1F on intermediate testing conditions defined in ICH Q1A (R2), a decision was made to retain 30°C/65%. However, regulatory authorities in the ICH regions have agreed that the use of more stringent humidity conditions such as 30°C/75% will be acceptable, if the applicant decide to use them (ICH guideline Q1A (R2), 2003).

History of discussions

At the 40th WHO Expert Committee Meeting, October 2005, the Committee determined that the WHO stability guidelines should be amended to reflect conditions for Zone III and IV as follows:

- Zone III: 30°C/35% RH
- Zone IVa: 30°C/65% RH
- Zone IVb: 30°C/75% RH

Further it was resolved that each individual Member State within the former Zone IV will need to classify itself as Zone IVa or Zone IVb.

On the 37th Report of The WHO Expert Committee on Specifications for Pharmaceutical Preparations, Geneva, 22-26 October 2001, it was discussed and adopted the recommended modification of storage conditions published in WHO guidelines for stability testing of pharmaceutical products containing well-established drug substances in conventional dosage forms to read 30°C (±2) and 65°C (±5%) RH for real-time stability studies destined for climatic zone IV. It was also agreed that where special transportation and storage conditions were identified as being outside these criteria, additional study data supporting these conditions should be available. For countries where certain regions are situated in Zone III or IV, and also with a view to the global market, it is recommended that stability testing programs should be based on the conditions corresponding to climatic Zone IV. In a stability study, the effect of variations in temperature, time, humidity, light intensity and partial vapor pressure on the product in question are investigated.

The effective or mean kinetic temperature reflects the actual situation better than the measured mean temperature; a product kept for 1 month at 20°C and 1 month at 40°C will differ from one kept for 2 months at 30°C (Markens, 2009; WHO Technical Report Series, no. 1010, Annex 10, 2018).

Moreover, the storage conditions are often such that the temperature is higher than the average meteorological data for a country would indicate.

Conclusion

A successful stability study will establish the shelf-life date on the drug product, the retest period of a drug substance and appropriate storage conditions—but that’s not all. A successful stability study must also ensure that patients receive a safe and effective medicine. To ensure that this happens, drug manufacturers need to thoroughly follow regulations and guidelines.

Acknowledgement

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Risk assessment on temperature and relative humidity deviation during on-going stability studies

Dragana Kafedziska*, Marina M. Micevska, Petranka P. Janoska, Elena Cvetanovska, Mena I. Zdravkovska, Milos Todorovski, Aleksandar Janusevski, Magdalena Blazevska, Hristina Babunovska

Alkaloid AD-Skopje, Pharmaceutical, Chemical and Cosmetics Industry, blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of Macedonia

Introduction

In ALKALOID AD Skopje, the stability test of commercial batches of pharmaceuticals is performed with on-going stability studies. Long-term tests are used for these studies, and samples for that purpose are stored, until expire date, in suitable walk-in chambers, under defined storage conditions: temperature (25°C ± 2°C) and relative humidity (60% RH ± 5% RH) (ICH, 2003). The defined storage conditions (temperature and relative humidity) must be maintained within the acceptable limits.

The aim of this work is to estimate the risk of temperature and relative humidity deviation in the walk-in stability chambers, taking measures for its control and reduction.

Materials and methods

The FMEA (Failure Mode Effects Analysis) method was used to perform this risk assessment (ICH, 2015).

FMEA provides an organized, critical analysis of potential failure modes of the system being defined and identifies associated causes (ICH, 2015). It uses occurrence and detection probabilities in conjunction with severity criteria to develop a risk priority number (RPN) for ranking corrective action considerations. RPN has been calculated on the basis of multiplication of the following indicators: severity, detection and probability.

Results and discussion

Quality risk management is a systematic process for the assessment, control, communication and review of the risks to the quality of the drug product across the product lifecycle (ICH, 2015; WHO, 2014).

Risk management methodology is based on the following:

Risk assessment

Risk identification – deviation of the defined storage conditions (temperature and/or relative humidity) is accomplished by monitoring systems. Identification of risk provides the basis for further steps in the quality risk management process.

Risk analysis – it is the qualitative or quantitative process of linking the likelihood of occurrence and severity of harms (ICH, 2015).
Sources of deviation of temperature and/or relative humidity might be:
- failure in the system for maintaining defined conditions;
- failure in the monitoring systems;
- inappropriate managing with walk-in chambers (e.g. inadequate door closing);
- inability to connect the generator to a voltage drop.

Risk evaluation – compares the identified and analyzed risk against given risk criteria. In case the defined conditions cannot be established in acceptable limits within 24 hours, the samples are transferred to another stability chamber with required performances. Corrective actions should be identified and implemented following the investigation to prevent reoccurrence (WHO, 2014).

The value of the RPN was estimated and calculated as 4.

Risk control

This phase includes decision making to reduce and/or accept the risk. The purpose of the risk control is to reduce the risk to an acceptable level. Established measures for risk control are:
- Continuous monitoring of temperature and relative humidity with two independent devices, MOGAL which is an integral part of the chambers and TESTO SAVERIS with its own separated probes and software.
- The values of the two monitoring devices are recorded at precisely determined and continuous time intervals. All records are documented and reviewed constantly.
- Monitoring alarms and alarms check - set points for alarms contain alert and action limits. The alert limits are set to allow preventive actions. These alarms alert relevant and trained persons in the form of SMS and e-mail messages, allowing timely detection of possible deviation.
- All processes related to chamber management, monitoring systems, documentation and recording of the values recorded by the two monitoring devices, calibration of the measuring instruments, chamber mapping are described with appropriate procedures and SOPs.
- Appropriate training should be provided for all staff members involved in the process of stability studies.

Additional risk management measures for risk control that are taken depending on the deviation background are:
- Temperature and relative humidity monitoring records are regularly reviewed and approved to ascertain whether a deviation may have occurred.
- Re-training should be provided in cases when deviation is due to inappropriate management with walk-in chambers.
- Re-calibration of sensors in cases where their validity is suspected.

Risk review

Once a quality risk management process has been initiated, that process should continue to be utilized for events that might impact the original quality risk management decision. The frequency of any review should be based upon the level of risk. Risk review might include reconsideration of risk acceptance decisions.

Conclusion

Based on the results of the risk assessment, it was concluded that the risk of temperature and/or relative humidity deviation in the walk-in stability chambers is considered as low. The benefit of this risk assessment is a reduction in the risk of deviation of storage conditions that prevents current stability studies from being compromised.

References

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Optimization and validation of HPLC method for determination of related and degradation products in Paracetamol tablets 500 mg

Aleksandra Damjanoska*, Cveta Dolikjoska Trajkova, Aneta Lazarevska Kamcheva, Nikola Pavleski, Hristina Babunovska

Alkaloid AD Skopje, Blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia

Introduction

Acetaminophen (N-acetyl-p-amino-phenol, AAP), also known as paracetamol, is a widespread antipyretic and analgesic accepted as an effective treatment for the relief of pain and fever in adults and children. As paracetamol could be hepatotoxic, the concentrations of the related constituents, 4-aminophenol (EP imp K, raw material residue), 4-nitrophenol (EP imp F, raw material residue) and 4-chloracetanilide (EP imp J, byproduct in API synthesis) should be strictly controlled, particularly the 4-aminophenol which is paracetamol main degradation product (Calinescu et al., 2012).

Although paracetamol has been available on the market for decades, new methods for determination of its impurities in dosage forms, following the current legislation, still need to be developed to ensure the safety of drug product. This paper reports the optimization and validation of a new, simple and reliable HPLC method for the simultaneous determination of impurities 4-aminophenol and 4-chloracetanilide in Paracetamol 500 mg tablets. The main challenge was to obtain good peak symmetry for the main component and achieve suitable level of quantification of 4-chloracetanilide, taking in consideration its extremely low specification limit of NMT 0.005%.

Materials and methods

Chemicals and reference standards

Potassium hydroxide, o-Phosphoric acid 85% and Acetonitrile were supplied from Merck KGaA, Darmstadt, Germany. Reference standard for Paracetamol was supplied from Alkaloid AD, Skopje; 4-Aminophenol and 4-chloroacetanilide were purchased from Merck KGaA, Darmstadt, Germany; 4-Nitrophenol was obtained from LGC Limited, England. The HPLC method has been validated to show specificity, linearity and range, accuracy, precision, limit of quantification (LOQ) and limit of detection (LOD), robustness, stability and filtration of solutions, as per ICH guideline (Q2 (R1) Validation of Analytical Procedures: Text and Methodology, 2005).

Instrumentation and analytical conditions

The analyses were performed on four different HPLC systems: Thermo Ultimate DAD 3000, Agilent 1260 Infinity Quaternary LC with UV-VIS detector, Nexera UHPLC DAD and Hitachi Chromaster 600 bar all controlled with Chromeleon CDS software version 7.2 SR5.
Results and discussion

The newly developed HPLC method for simultaneous determination of 4-aminophenol and 4-chloroacetanilide was optimized by using column Zorbax Eclipse Plus C18; 250 x 4.6mm, 5 µm; with column temperature of 25 °C, autosampler temperature of 4 °C, at a 1 mL/min flow rate and 245 nm detection. The injection volume was 10 µL. Solution A (phosphate buffer pH=6.3) : Acetonitrile (ACN) gradient method was set: 0–12 min 10% ACN, 12–38 min from 10% to 30% ACN, 38–58 min 30% ACN, 58–60 min from 30% to 10% ACN, and 60–65 min 10% ACN.

The system suitability was evaluated on the basis of resolution between 4-aminophenol and paracetamol peak (Resolution, Rs>5.0). There was no interference from diluent and placebo with paracetamol, 4-aminophenol and 4-chloroacetanilide, indicating the specificity of the method. Linear correlations were obtained between the responses of 4-aminophenol peak related to the concentrations of standards over the range of 0.0005% (0.025 µg/ml) – 0.3% (15 µg/ml), (r=1.00), paracetamol peak related to the concentration of standards over the range of 0.0005% (0.025 µg/mL) – 0.3% (15 µg/mL), (r=1.00), and 4-chloroacetanilide peak related to the concentration of standards over the range of 0.0005% (0.025 µg/mL) – 0.3% (15 µg/mL), (r=1.00). The accuracy of the method was evaluated using an equivalent amount of placebo present in paracetamol tablets 500 mg at working concentration spiked with known quantities of 4-aminophenol, paracetamol and 4-chloroacetanilide at five different levels, in triplicate. The samples were analyzed by the proposed method and the amount of 4-aminophenol, paracetamol and 4-chloroacetanilide recovered was calculated. All the obtained results were into the range of the acceptance criteria for recovery (90.0-110.0%) and RSD (≤10%). The precision of the method was verified by repeatability and intermediate precision. The repeatability was shown by six replicate injections of the standard solution containing 4-aminophenol in the working concentration of 5.0 µg/mL, paracetamol in the working concentration of 10.0 µg/mL, and 4-chloroacetanilide in the working concentration of 0.5 µg/mL.

The intermediate precision of the method was evaluated using different analyst, column and different instrument and the analysis was performed on different days. The LOD and LOQ for impurities were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of diluted solutions with known concentrations (for paracetamol and both impurities LOD is 0.01 µg/mL and LOQ is 0.03 µg/mL). The method was robust for all the varied conditions. Standard solutions of 4-aminophenol at concentration level of 5.0 µg/mL, paracetamol at a concentration level of 10.0 µg/mL, 4-chloroacetanilide at a concentration level of 0.5 µg/mL and sample solutions prepared as per test method were analyzed initially and at different time intervals (24 h, 48 h, 72 h, 96 h, 120 h) by keeping the solutions at temperature of 4 °C and protected from light. From the obtained data, it was concluded that the standard solution is stable for 96 hours and sample solution is stable for 72 hours at temperature of 4 °C and protected from light. The results from the filter study indicated that no differences are observed when the standard solution is filtered through RC filter (0.45µm pore size) and PVDF filter (0.45 µm pore size).

Conclusion

It can be concluded that the defined RP-HPLC method is rapid and efficient for purity testing of commercially available Paracetamol 500 mg tablets.

References

Regulatory approaches for medicines containing established active substances in USA

Bojana Danilova¹*, Jasmina Tonic Ribarska², Suzana Trajkovic Jolevska², Katerina Brezovska², Jelena Lazova¹

¹Alkaloid AD Skopje, Blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia
²Faculty of Pharmacy, Ss Cyril and Methodius University, 1000 Skopje, Republic of North Macedonia

Introduction

The term “Established Active Substance” refers to an active pharmaceutical ingredient (API) that is identical to an API already in a drug product, which is approved for marketing in the United States of America (USA) under section 505 of the Federal Food, Drug, and Cosmetic Act (FD&C Act).

In this study, the regulatory requirements for the medicines containing established active substances and possible regulatory approaches for line extensions and generics have been studied in line with the USA governing laws and regulatory environment. The aim is to critically overview the use, the requirements and the outcome of different regulatory procedures suitable for the abridged applications.

Regulatory background

The Federal Food, Drug, and Cosmetic Act (FD&C Act) and the FDA Rules and Regulations published under Title 21 of Code of Federal Regulations as well as the effective Guidelines for industry, information available in the official FDA website and published journal articles have been reviewed in order to collect data and develop a regulatory strategy for medicines containing established active substances.

* bdanilova@alkaloid.com.mk

Once the New Molecular Entity NDA (New Drug Application) is approved by FDA under the section 505(b)(1) of the FD&C Act, the medicines containing established substances options for submissions are as follows:

SNDA – Supplemental New Drug Application

This application can be submitted only by the NDA owner, as it relates to changing the already approved NDA, which may result with line extensions. Subject to SNDA can be changes for which prior approval is required, including changes in the drug substance, drug product or labelling (i.e. change in composition, indications, dosing regimen, additional strength, form etc.) and changes for which prior approval is not required (i.e. tightening the manufacturing controls, safety changes in labelling, etc.), also known as “Changes Being Effected”.

ANDA – Abbreviated New Drug Application

This type of application is filed for generic drug ‘copy’ of an existing licensed medication. The basis for ANDA were established by the “Drug Price Competition and Patent Term Restoration Act of 1984”, also known as the “Hatch-Waxman Act”.

The exclusion of the pre-clinical and clinical trials, the introduction of bioequivalence study only, the reduction of time and cost for placing generic drugs
on the market, Roche-Bolar provision, are just a few of the many significant results due to this Act. According to the 21CFR314.92, the drug product eligible for ANDA should be same as the Reference Listed Drug (RLD) chosen by FDA in terms of active ingredient(s), strength, dosage form, route of administration, as well as quality and performance characteristics, bioavailability and intended use. For drugs that differ from the RLD, Suitability Petition under the section 505(j)(2)(C) from the FD&C Act should be submitted in order FDA to determine if the product is eligible for ANDA. ANDA data requirements include bioequivalence study (must demonstrate BE to RLD), CMC data (complete quality dossier and comparable dissolution data vs RLD) and Labelling (same as RLD, minus exclusivity). ANDA must include Patent certifications for each patent listed in the “Orange Book” for the RLD. The first generic drug approved under patent certification for paragraph IV (claiming that the patent of the RLD will not be infringed by the ANDA), gains 180 days of market exclusivity. With implementation of Generic Drug User Fee Act (GDUFA), the standard review time has been shortened to 10 months. The priority review is reserved for first ANDAs, market shortages etc. and the review time goal is set to 8 months.

505(b)(2) NDA

“Hatch-Waxman Act” has also established basis for approving drugs by relying on literature studies and previous public FDA findings, avoiding unnecessary duplication of studies already performed. This application contains “full reports of investigations of safety and effectiveness, where at least some of the information required for approval comes from studies not conducted by or for the applicant and for which the applicant has not obtained a right of reference or use”. A ‘hybrid’ procedure that requires a bridge between the new version of the product and the RLD(s). This could include data and results of bioanalytical testing, preclinical studies, or even clinical trial results. These drugs are not strictly generics, but are often not entirely novel new molecular entities either. 505(b)(2) candidates are drugs with a new aspect related to indication, dosage form, strength, formulation, dosing regimen, route of administration, dispensing regimen (Rx-OTC), new combination, prodrugs of an existing drug, and in some cases, drugs with new API (different salt). This pathway is not appropriate for duplicates of RLD or if the only difference from the RLD is decreased rate or extent of absorption. Appealing aspect is that the 505(b)(2) approved drug is eligible for 3, 5 or 7 years of market exclusivity. According to Prescription Drug User Fee Act (PDUFA), the standard review time is 10 months, and a 6 months goal is established for priority review NDAs.

OTC applications

If the OTC drug complies with the corresponding FDA OTC monograph, no FDA approval is needed to market the drug. If the OTC drug does not comply with a OTC monograph, then the following applications should be considered: OTC NDA, OTC 505(b)(2) NDA, OTC ANDA or SNDA of an existing Rx NDA. The Time and Extent Application (TEA) under the section 21CFR 330.14 is another mechanism to incorporate a "new product or a product condition" in a monograph. This two-step process includes review of condition eligibility for inclusion in the OTC drug monograph system, followed by safety and effectiveness data submission for the determined conditions as eligible.

Summary

When determining the appropriate regulatory strategy for approval of a product that contains established active substance(s), it is important firstly to identify the proposed product characteristics with respect to the listed drug(s). The exact RLD ‘copies’ are suitable for ANDAs. The diverse products from RLD, may be suitable for 505(b)(2) NDA or ‘Petitioned ANDA’. If OTC, consider marketing per monograph or submitting NDA/ANDA.

References


Some challenges in global clinical development of generic products

Rozeta Mileva Peceva1*, Jasminka Patcheva2, Snezana Petrovska1

1Alkaloid AD Skopje, Blvd. Aleksandar Makedonski 12, 1000, Skopje, R.N. Macedonia
2Pharmaceutical Chamber of Macedonia, 50-ta Divizija, 1000, Skopje, R.N. Macedonia

Introduction

The regulatory agencies globally define the regulatory requirements for the approval of simple small-molecule generic products (Hornecker et al., 2009). Following the globalization of the pharmaceutical supply chain, from clinical trials to drug development and manufacturing, the harmonization of pharmaceutical regulations is essential. Significant progress on industry globalization without regulatory harmonization impacts manufacturers and patients (Alvaro et al., 2019). Many regulatory agencies have limited resources, are lacking sufficient skilled staff and are challenged to stay abreast of rapid advances in new treatments and new technologies (Elias and Hamburg, 2016). Meanwhile companies are often required to conduct similar but distinct studies and submit multiple application for given product to agencies in different countries, increasing the time and the cost it takes to bring new drug to market (Weisfeld and Lustig, 2013). For example, in a case of a generic application when supportive bioequivalence studies are needed, FDA lists as RLD (reference listed drugs) the product which is to be used as a comparator in the bioequivalence study. However, even if the same medicinal product is to be used in a bioequivalence study for EU submission, it must be purchased from the EU market. On the other hand, Canada accepts a drug product purchased in another country which complies with the certain criteria to be considered acceptable to the Minister for use as a Canadian Reference Product (HC, 2017). A solution in such scenario is that generic companies conduct separate bioequivalence studies for each region.

Aims and objectives

This paper has intention to point out some similarities and differences in the recommendation of EMA, FDA and Health Canada (HC) on some aspects on bioequivalence studies, which affect the generic industry during clinical drug development process of oral immediate release formulations.

Materials and methods

Analysis of several aspects on the requirements for the bioequivalence studies for oral immediate release formulations of relevant guidelines from of EMA, FDA and HC has been conducted. The following Guidelines were analyzed:
1. FDA, Guidance for Industry Bioavailability and Bioequivalence Studies for Orally Administered Drug Products – General Considerations. 2003;
2. FDA, Bioavailability and Bioequivalence Studies Submitted in NDAs or INDs — General Considerations. Draft Guidance. 2014;
3. EMA, Committee for Medicinal Products for Human Use. Guideline on the Investigation of

* rozeta.mileva@gmail.com
Results and discussion

The results of the analysis have shown that although the analyzed Guidelines from the 3 regions (EU, USA and Canada) are consistent regarding the recommendation to conduct the bioequivalence studies in healthy subjects above 18 years old (in case there are no safety issues), there are differences regarding several aspects, including subject’s gender and race. Unlike FDA Guidelines, where the study population should be representative of the general population in terms of age, sex and race the EMA and Health Canada Guideline do not specify the gender and race of the study subjects. Furthermore, according to FDA recommendation, the generic drug product should be generally tested under both fasting and fed conditions. On the other hand, in order to fill in for an application to EU or Canada, the dossier of an immediate release product may be supported generally with one bioequivalence study under fasting conditions, in case the drug is taken under an empty stomach or irrelevant to food intake. As for the sample size, the EMA and HC guideline sets the minimum required subjects at 12 completers, whereas FDA instructs for adequate statistical power for a BE demonstration. According to EMA guidance, the over-night fasting time is at least 8 h, the same as HC, and the volume of fluid to be taken with the treatments is identified as at least of 150 mL (EMA, 2010). FDA recommends at least 10 hours overnight fasting before drug administration with 240 mL of water, and as per HC, the medication is to be taken with 150 to 250 mL of water and at a standard temperature.

Conclusion

The present article has highlighted some similarities and differences in the requirements of the guidelines on bioequivalence between FDA, EMA and HC. There are some similarities, but there are also differences in several aspects. Facing the fact that it is difficult to predict global regulatory landscape in the near future, the need of pragmatic and step-wise approach in harmonization is more than necessary. The generic industry should be aware for the above at early stage of drug product development, in order to create appropriate clinical strategy and save resources.

References


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Social aspect as a part of HRQoL in patients with cystic fibrosis in Republic of North Macedonia

Zoran Nakov1*, Stojka Naceva Fushtikj2, Stevce Acevski3, Jasmina Tonic Ribarska4, Suzana Trajkovic Jolevska4

1Novo Nordisk Pharma DOOEL, st. Nikola Kljusev 11, 1000 Skopje, Republic of North Macedonia
2University clinic for children diseases, Ss. Cyril and Methodius University, Mother Tereza 17, 1000 Skopje, Republic of North Macedonia
3Alakloid AD, blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia
4Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Tereza 47, 1000 Skopje, Republic of North Macedonia

Introduction

Cystic fibrosis (CF) is a genetic disorder that occurs as a result of mutations in a single gene responsible for synthesis of a protein known as a cystic fibrosis transmembrane conductance regulator (Tsui and Buchwald, 1991). CF is a rare disease and according to available data in 33 EU countries, more than 42000 patients with CF have been registered. In Republic of North Macedonia (RNM), the average number of patients with CF is 110. Considering the fact that more than 20 patients of the whole population of RNM are affected with CF, this disease is not administratively recognized as a rare disease in our country.

The clinical picture of CF is dominated by symptoms of impaired functioning of lungs, liver, kidneys and intestine too (Naceva Fushtikj, 2012). The medical treatment of CF includes inhalation of mucolytic, inhalation of antibiotic, enzyme, vitamin and physical therapy. The limitations that patients experience due to the daily practice of the necessary therapy, inevitable affects their social activity.

The main objective of this study was to assess the current social aspect of patients with CF in RNM. The measurement of the social aspect of patients with CF is important in order to improve their functional capabilities. The social aspect refers to the quantity and quality of social contacts and interactions (Goodman, 1998).

The assessment of the social aspect was conducted as a part of Health-related quality of life (HRQoL), which provide the patient’s general subjective perception of the effect of illness and intervention on physical, psychological and social aspects of daily life (EUnetHTA, 2013).

Materials and methods

The participants included in this research were adults and adolescents older than 13 years. The survey of the patients was conducted by using the Macedonian version of Cystic Fibrosis Questionnaire Revised (CFQ-R) as a disease-specific HRQoL instrument. The questionnaires were administrated as a self-administered. All involved patients were informed about the study objectives and data confidentiality, and were asked to indicate their agreement to participate.
The answers of the questions from HRQoL module were given as a 5 distinct 4-point Likert scales (always/often/sometime/never). The score was ranged between 0 to 100, whereas higher scores representing a better health. The statistical analysis was performed using SPSS statistical software, Student t-test for independent samples, Mann-Whitney nonparametric test, Kruskal-Wallis ANOVA-post hoc Mann-Whitney test, Pearson test and Spearman test.

Results and discussion

The HRQoL survey included thirty-one patients (adults and adolescents) and 12 domains of HRQoL (symptoms and health status) were analyzed. Among them there were 20 men and boys (64.52%) and 11 women and girls (35.48%). The ethnic structure of the patients was dominated by ethnic Macedonians (23 ethnic Macedonians, 5 ethnic Albanians, 1 other and 3 preferred not to respond).

The questions included in the questionnaire intended for assessment of socials aspect of life were connected to the ability of patients for regular social activity on a daily basis.

The Macedonian patients in comparison with Albanian patients reported higher score for social activity, but there was no statistically significant difference. Taking into account the fact that Macedonian and Albanian patients belong to the white race, differences in the manifestation of symptoms of CF, and consequently the impact of the disease on the social aspect is not expected. The male patients scored their social activity (p=0.43) not significantly higher compared to female patients.

The social activity was reported with the mean score of 49.64. The obtained score for the social activity was the lowest among the other 11 parameters measured with the HRQoL (emotion, vitality, physical condition, respiratory, eating, digestion, weight, body image, treatment burden, health perception and school/work performance).

This finding may be explained with the fact that 70.97% of the surveyed patients are teens and young adults (between 13 to 25 years of age) when it is normally to exist a negative impact of their medical condition in their self-perception and in the execution of their daily social activities. The negative impact of CF in execution of daily patient’s social activities was also reported by Gee et al. (2003). Low score for social aspect was also obtained in our previous reported study for the HRQoL in pediatric patients in RNM (Nakov et al., 2019).

Conclusion

The lowest scores for social activities among other 11 investigated parameters shows that this medical condition has a negative impact on the patients’ self-perception and in the execution of their daily social activities.

The adherence of the patients to the regular CF treatment regimens, which include medication and physical therapy and physical activity, is inevitable accompanied with shortness of time for their daily social activities.

This fact is main reason why these patients scores their social life with the lower score.

References

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Implementation of the new EMA methodology of defining risks and the HaRP methodology in the EU Risk Management Plan for Ibuprofen 40 mg/mL oral suspension

Vladimir Kostovski*, Irina Dukovska, Ivona Trpenoska Aleksovska, Ace Kuzmanovski, Sofija Dimkoska, Marija Crcarovska, Natasha Vukikjevikj

Global Pharmacovigilance Unit, Alkaloid AD, Blvd. A.Makedonski 12, 1000 Skopje, North Macedonia

Introduction

Risk is associated with every single aspect in pharmaceuticals from development of a molecule to its way to the customer (Trivedi et al., 2016). The purpose of the risk management plan (RMP) is to document the risk management system, which is a series of pharmacological activities for identification of the risk, its assessment, minimization or prevention and its communication (Garlapati and Nagandla, 2015). In EEA submission of RMP is required at the time of application for a marketing authorization of a medicine and additionally whenever information affecting the benefit-risk balance of a medicine is available.

After releasing the majority of Good Pharmacovigilance Practices (GVP) guidelines in 2012, The European Medicines Agency (EMA) committed to continuously improve the PV legislation (von Bruchhausen and Schirp, 2017). In early 2016, EMA started a major revision process of the good pharmacovigilance practice (GVP) Module V on risk management systems.

The final guidance, released at the end of March 2017, set a new milestone in the process of continuous improvement of the RMP guidance. The second revision of GVP module V provided guidance on the reconsideration of safety concerns from the existing RMP in the post-authorization period (EMA, GVP V, 2017). This encouraged marketing authorization holders (MAHs) to critically revise the list of safety concerns during preparation of the RMP, pharmacovigilance activities, and risk minimization measures in the post-marketing phase.

Revision 2 clarifies that risks should be identified through adverse clinical outcomes that are caused by the use of a medicinal product (identified risks) or that might be caused by the use of a medicinal product (potential risks). The important potential risks are those, when further characterised and if confirmed, would have an impact on the risk-benefit balance of the medicinal product (von Bruchhausen and Schirp, 2017). Important potential risks can be removed from the RMP when they are not being considered important or when there is no reasonable expectation that any pharmacovigilance activity can further characterize the risk, or they can be reclassified to important identified risks.

Materials and methods

Methodology of HaRP (Harmonisation of RMP Project)

The Coordination Group for Mutual Recognition and Decentralised Procedures - Human (CMDh) in June 2019 have published the document Annex 2:
HaRP, which aim was to harmonise the RMPs of the same active substances for which marketing authorisations have already been granted with different RMPs in place. First review and harmonisation of the list of safety concerns is performed by the HaRP peer review group and is based on those RMPs published on the Excel list.

Following this initial review, in a subsequent consultation round, feedback is asked from Pharmaceutical Industry and all Member States. The active harmonization can be implemented in two domains. Domain 1 - RMPs for which data exclusivity of reference product will expire. In this domain, the RMP of the reference product will be assessed in the context of revision 2 of GVP Module V and post-marketing experience with the product to date. Domain 2 referred to clean-up of the List of safety concerns as published on the CMDh website (first step for substances with no reference product or with reference product without a RMP in place). In domain 2, an additional algorithm has been agreed to harmonise the list of safety concerns. For active substances for which there is no innovator or the innovator has no RMP, only those safety concerns should be listed that either have: ongoing additional pharmacovigilance activity, ongoing additional risk minimisation measure, or essential targeted questionnaires in place. In July 2019, CMDh published HaRP assessment reports for twenty-one medicines (CMDh, HaRP, 2019). The new methodology of defining risks and the principles from the HaRP methodology were implemented during preparation of the EU RMP for Ibuprofen 40 mg/ml oral suspension.

Results and discussion

The active substance of the medicinal products Ibuprofen 40 mg/mL oral suspension, (ibuprofen), falls under Domain 2. The proposed important identified risks for ibuprofen in the initial version of the RMP were: gastrointestinal bleeding, cardiovascular events, hepatic and renal disorders, use during pregnancy and severe skin reactions; important potential risk was impaired fertility and proposed missing information were breast feeding and use in children under 6 months of age and under 7 kg body weight.

Ibuprofen is well known active substance and the safety concerns are already known to the healthcare professionals and adequately described in the product information. For all safety concerns for ibuprofen according to the HaRP methodology there were no ongoing additional pharmacovigilance activity, ongoing additional risk minimisation activity, ongoing additional risk minimisation measures and no essential targeted questionnaires in place. Therefore, in the final version of the document according to the new methodology and the algorithm, all safety concerns were deleted and the summary of safety concerns was published without risks. This approach and the document itself were accepted by the dedicated regulatory agencies in EU, thus the procedure ended with positive outcome.

Conclusion

RMP is dynamic document that is continually updated throughout the life cycle of the product and when significant information is available. Revision 2 of GVP module V resulted with simplification and shortening of the Risk management plan (von Bruchhausen and Schirp, 2017), which is also result of using the HaRP methodology, as was the case with the medical writing of RMP for Ibuprofen 40 mg/mL oral suspension. The focus of the revised RMP is on identifying or characterizing the safety profile of the medicinal product, proposing measures to prevent or minimize the risks, and including an assessment of the effectiveness of the proposed measures. Expectation is that over time the additional pharmacovigilance activities in a certain RMP in which they are included, will be completed and thus removed from the RMP, or the additional risk minimization activities will be evaluated and modified if necessary throughout the whole life cycle of the product.

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Quality-by-design based development of a stability indicating method for antiviral ophthalmic ointment

Emilija Arsovska Popovska*, Filip Gogu, Nina Peneva, Frosina Jovanovic, Eleonora Trajanovska, Suzan Memed Sejfulah, Ana Atanasova, Packa Antovska, Sonja Ugarkovic

Institute for Research and Development, Alkaloid AD-Skopje, Blvd. Aleksandar Makedonski 12, 1000 Skopje, North Macedonia

Introduction

Some ocular pathologies such as herpes simplex, virus retinitis and acute retinal necrosis are usually treated by administering antiviral drug product. Antiviral eye ointment is a sterile preparation, manufacturing by incorporation of very finely powdered antiviral active substance into a petrolatum base. The base is designed to have melting point close to human body temperature and can be used as anhydrous medium for the delivery of moisture-sensitive drugs.

Several methods have been reported for the determination of this active component in pharmaceutical formulations, based on different analytical techniques. HPLC method for the assay of active compounds is superior to other conventional methods in speed, precision, specificity and ease of performance.

The aim of our work was to develop rapid HPLC assay method for determination of antiviral active compound by implementation of QbD principles. Quality by design approach in method development help to better understand chromatographic variables in less time compared to traditional, one factor at a time method development (Gayakwad et al., 2015; Karmarkar et al., 2011).

Materials and methods

All the chemicals and reagents used in this study were of analytical grade with high percentage purity. Active substance standard, analytical reference standards and related impurities were supplied from EDQM.

Working solutions of drug product were prepared according to in-house method, in concentration 0.025 mg/mL. According the method, eye ointment should be suspended in nonpolar organic solvent at 60 °C, and target compound isolated by extraction with aqueous solvent.

The development and optimization were performed on Agilent 1290 Infinity HPLC system supplied with binary pump connected to a 12-position solvent selection valve and two thermostated column compartments with 8-position valves allowing eight columns to be coupled at the same time. Chromeleon Console chromatographic software was used to control the instrument, compile data and process the same.

Statistical experimental design, data processing, modelling and optimization of the analytical method were accomplished using Fusion AE (version 9.7.1 SR2c, S-Matrix Corporation, Eureka, CA), which is QbD based software for analytical method development that integrates method robustness testing into method development. Fusion method

*earsovska@alkaloid.com.mk
development is two phase strategy; composed of screening and optimization phase. Screening experiments examine the critical chromatographic selectivity factors, including column type, pH and mobile phase composition. Method optimization phase use the results from screening phase plus additional variables with tighter ranges to determine the optimum chromatographic conditions.

In the screening phase following conditions were tested: ten different columns with C18 chemistry of stationary phase: Inertsil ODS-3V 250-4.6 mm (5 µm), ACE 5 C-18 250-4.0 mm (5 µm), Zorbax Eclipse Plus C18 250x4.6 (5 µm), XBridge C18 150-4.6 mm (5 µm), X-Terra RP18 150-4.6 mm (5 µm), SunFire C18; 150-4.6 mm (3.5 µm), XBridge C18 150-4.6 mm (5 µm), Inertsil ODS-3V 150-4.6 mm (5 µm), BDS Hypersil 100-4.6 mm (3 µm); two types of organic solvents: methanol and acetonitrile, three different buffer types containing TEA, CH$_3$COONH$_2$ or KH$_2$PO$_4$ and different range of pH of the buffer: pH 2.5, pH 4.5 and pH 6.5. Flow rate was tested in range 0.8 mL/min–1.5 mL/min, in isocratic mode. Injection volume was 10 µL. UV detection was performed at 254 nm.

**Results and discussion**

According to obtained results, analyzed with Pareto diagram and graphical reports, two key parameters have main influence on chromatography, type of column and type of organic modifier, while other experimental variables, type of buffer, pH and flow rate have minimal effect.

Due to hydrophilic nature of the active component, retention characteristics of the solute are affected by minimal volume percent of organic modifier in mobile phase. When acetonitrile is used as organic modifier in the mobile phase, active compound has very little retention on all columns tested. The retention of components was significantly better by using methanol as organic modifier. Since our purpose is to develop robust and reproducible method with reasonable percent of organic modifier, methanol was chosen as organic solvent in mobile phase for next stages of method optimization.

Inertsil ODS-3V 250-4.6 mm (5µm) column provides broad range of combinations of screening conditions (percent of organic solvent and flow rate) at which the specified criteria meet the set goals, hence it is selected for next method optimization step. In method optimization phase finer variations of experiment conditions were tested: narrower range of pH (pH 2.3–pH 3.2), proportion of methanol (10%-20%) in mobile phase, flow rate (0.8 mL/min to 1.5 mL/min) and column temperature (25 °C – 40 °C).

Final conditions for the analytical method were chosen by application of Fusion method development overlay graph, which predicts the ranges of chromatographic parameters that generate robust method. After confirming optimized method for quantitative determination of active compound in the presence of its impurities and degradation products, specificity, linearity, accuracy, robustness and precision were confirmed with method validation as per ICH guidelines.

**Conclusion**

Comprehensive analytical method for quantification of antiviral drug product was developed by application of automated QbD method development approach using Fusion AE software. Multivariate analysis of several critical method parameters including column and solvent type, mobile phase composition, pH, column temperature and flow rate were used to determine the best performing analytical procedure. A robust final method was obtained with a column temperature of 25 °C, percent strong solvent of 15%±2%, and pH 2.5±0.2. QbD principle to method development and optimization has enabled to better understand the method variables, leading to less chance of failure during method application. The implementation of QbD Fusion AE approach has provided a better performing and more robust method in less time compared to manual method development.

**References**


Monitoring the changes in ALP, AST and LDH activity during short-term orthodontic treatment using multivariate algorithms for chemometric data analysis

Liljana Anastasova¹*, Angela Tasevska², Natasa Toseska Spasova², Mirjana Popovska³, Rumenka Petkovska¹

¹Institute of Applied Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy, Ss. Cyril and Methodius University, 1000 Skopje, Republic of North Macedonia
²Department of Orthodontics, Faculty of Dentistry, Ss. Cyril and Methodius University, 1000 Skopje, Republic of North Macedonia
³Department of Oral Pathology and Periodontology, Faculty of Dentistry, Ss. Cyril and Methodius University, 1000 Skopje, Republic of North Macedonia

Introduction

Chemometrics is a science of multidisciplinary nature which has a widespread application in different fields. In medicine and pharmacy, chemometric algorithms for multivariate data analysis, among other applications, are used to monitor patient’s health as well the effects of the medical treatments (Mocak, 2012). Short-term orthodontic treatment consists of application of orthodontic force in order to produce tooth movement. Monitoring the activity of certain biomarkers in gingival crevicular fluid (GCF) could be a clinically useful and noninvasive procedure to assess the changes in periodontal tissues after orthodontic force application (d’Apuzzo et al., 2013; d’Apuzzo et al., 2017). Several enzymes in GCF, such as alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) have been assessed during orthodontic treatment and could be used for monitoring tissue changes during treatment (d’Apuzzo et al., 2013; Perinetti et al., 2012). The aim of our study was to examine changes in the ALP, AST and LDH activity in GCF during the short term orthodontic treatment using chemometric algorithms for multivariate data analysis.

Materials and methods

The study protocol was approved by the Ethics Committee at the Faculty of Dentistry, Skopje. Twenty orthodontic patients aged from 13-28 were enrolled in the study. Informed consent was obtained from the patients after providing information about the study. Fixed orthodontic appliance, Roth 22 Dentaurum was used. GCF samples were collected from each patient before application of orthodontic force (t0) and after 7 (t1), 14 (t2) and 21 days (t3) of the treatment, from the mesial and distal side of the maxillary test canine. Four paper strips were inserted in the gingival crevice and left in place for 30 s (Koss et al., 2009). Afterwards, they were transferred in eppendorf tubes containing 250 μL phosphate buffer saline (PBS, pH=7.4) and stored at -20 °C until analysis. ALP, LDH and AST activity in GCF samples was determined using commercially available kits, according to manufacturer’s instructions. Chemometric algorithms for multivariate data analysis, principal component
analysis (PCA) and the orthogonal projections to latent structures discriminat analysis (OPLS-DA) were applied using SIMCA 14.1 software (Umetrics, Umea, Sweden). The obtained data from ALP, AST and LDH activity during short term orthodontic treatment was divided into mesial data set and distal data set and pareto-scaled for multivariate data analysis.

Results and discussion

PCA was applied on the two datasets to investigate global data variability. Several outliers were detected for each data set and removed from further analysis. The OPLS-DA algorithm provides comprehensive description on the discrimination between classes of samples, especially in cases where subtle differences among classes are present (Bylesjo et al., 2006). This algorithm separates the systematic variation in the matrix X (ALP, AST and LDH activity in GCF in the selected time intervals) in two parts, one linearly related (variation of interest) to the matrix Y (the classification variables) and one orthogonally related (so-called orthogonal variation or structured noise) to the matrix Y. OPLS-DA analysis was applied to investigate differences for each time interval and t0. The data for the biomarker activity at t0 and t1, for both distal and mesial data sets were overlapped. This finding might be due to time needed the “stressed” tissue to respond to mechanical stimuli. The score plots of OPLS-DA models comparing biomarker activity at t0 and t2, for both mesial and distal data sets resulted in clear separation of two groups. The cross validation parameters for OPLS-DA model for the mesial data set were $R^2_X(cum) = 1$, $R^2_Y(cum) = 0.579$ and $Q^2_X(cum) = 0.513$, indicating a good model. Based on the VIP statistics from the cross-validated OPLS – DA model, statistically significant variables responsible for separation between the two treatment points (VIP value > 0.8) were extracted. The most important variables responsible for separation in the mesial data set were ALP and AST activity. There was also clear separation between groups for the distal data set containing biomarker activity at t0 and t2, with the following model characteristics: $R^2_X(cum) = 1$, $R^2_Y(cum) = 0.404$ and $Q^2_X(cum) = 0.419$. According to the VIP criterion, ALP and AST activity in GCF were the most important variables for class separation. The comparison of biomarker activity at t0 and t3 for the mesial data set resulted in two distinct clusters of data and satisfactory model characteristics: $R^2_X(cum) = 0.803$, $R^2_Y(cum) = 0.457$ and $Q^2_X(cum) = 0.415$. The most important biomarkers responsible for class separation were the ALP activity and AST activity. The score plot of the OPLS-DA model for the last time point of the distal data set also gave two groups and good model statistics: $R^2_X(cum) = 1$, $R^2_Y(cum) = 0.438$ and $Q^2_X(cum) = 0.401$. However, compared to the mesial data set, the most important variables responsible for class separation were ALP activity and LDH activity, whereas AST activity had no influence on class separation.

Conclusion

Multivariate chemometric data analysis was applied as an approach to evaluate changes in ALP, AST and LDH activity during short-term orthodontic treatment. This approach may be used as means to assess treatment progress or to avoid adverse effects of treatment on the periodontium.

References

Collection, sample preparation and analytical methods for determination of therapeutic levels of drugs in gingival crevicular fluid – a review

Liljana Anastasova¹*, Mirjana Popovska², Rumenka Petkovska¹

¹Institute of Applied Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy, Ss. Cyril and Methodius University, 1000 Skopje, Republic of North Macedonia
²Department of Oral Pathology and Periodontology, Faculty of Dentistry, Ss. Cyril and Methodius University, 1000 Skopje, Republic of North Macedonia

Introduction

Gingival crevicular fluid (GCF) is an inflammatory exudate containing substances from the host (enzymes, cytokines, interleukins or tissue breakdown products), as well as from supra- and subgingival located bacteria (Wassall & Preshaw, 2016). It plays a significant role in the oral defense mechanism and facilitates transport of antibacterial substances into the periodontal pocket. Literature findings imply that certain drugs used in the treatment of periodontal disease can accumulate in gingival fibroblasts and serve as reservoirs which gradually release the substance in GCF. Therefore, GCF concentrations of certain drugs can be used as an indicator of treatment response (Dincel et al., 2005; Lai et al., 2011). This review will provide an overview of the methods for GCF collection, sample preparation, commonly used analytical methods for quantitative determination of drug concentration in GCF as well as future developments in this area.

Collection methods and sample preparation techniques for GCF samples

The collection of GCF samples is regarded as noninvasive and relatively simple procedure without having to take a sample from gingival tissue. However, GCF volumes are typically low (microliter quantities) and generally increase with increasing inflammation in gingival and periodontal tissues. Therefore, the quantity and quality of GCF samples can be highly affected by the method of collection and analysis. The GCF sampling procedure has been performed using a variety of methods, including gingival washing techniques, capillary tubes or micropipettes as well absorbent filter paper strips. The most frequently used technique is the filter paper method (Wassall & Preshaw, 2016). In the last decade, researchers have favored the filter paper strips due to easy insertion into a gingival crevice up to 1 mm depth without bleeding from periodontal pockets (Khurshid et al., 2017).

GCF is a complex matrix especially in inflamed sites where the protein composition resembles the serum (Papagerakis et al., 2019). In order to minimize the interferences that could arise in the determination of the drug concentration in GCF, prior analysis liquid-liquid extraction usually followed by vortexing or centrifugation is performed. In this manner, the drug of interest is eluted in the supernatant and ready for subsequent analysis (Dincel et al., 2005; Sagan et al., 2005).

* lbogdanovska@ff.ukim.edu.mk
Methods for quantitative determination of drugs in GCF samples

For the quantitative determination of drug concentrations in GCF several analytical methods have been employed: gas chromatography (GC), high-performance liquid chromatography (HPLC) as well as high-performance liquid chromatography coupled with mass detection (HPLC-MS).

GC analysis has been applied for determination of nifedipine which is sequestered into GCF causing drug-induced gingival overgrowth. The main disadvantage of this “scenario” is the derivatization step in the sample preparation procedure in order to make it suitable for GC analysis (Guncu et al., 2007). HPLC is a powerful tool in the analysis of drugs in different biological matrices. It has the ability to separate and identify compounds that can be dissolved in suitable liquid and detect trace concentrations as low as parts per trillion. Also, the wide pallet of detectors available for the HPLC enables determination of different molecules (Dong, 2013). HPLC with UV detection has been used in the analysis of drugs such as metronidazole, tinidazole, phenytoin, spiramycin and other commonly used antibiotics especially in the treatment of aggressive forms of periodontal disease. Another group of commonly applied drugs in periodontal treatment, both locally and orally, are fluoroquinolones and tetracyclines. They are accumulated by fibroblasts in gingival connective tissue which potentially enhances their antimicrobial effectiveness in two ways: by increasing their local concentrations in gingiva and sustaining them for a longer duration, which is a factor that must be considered in order to optimize periodontal antimicrobial chemotherapy (Lavda et al., 2004).

Due to the specific chemical structure of these molecules HPLC with fluorescent detection has been used as the method of choice in their analysis. In this manner greater sensitivity is achieved (Dincel et al., 2005; Lavda et al., 2004).

Another recently applied method for quantitative determination of drugs in GCF is LC-MS/MS. Compared to HPLC-UV it requires smaller sample volume, it has better specificity and a higher speed of analysis (Sagan et al., 2005). However, due to the complex equipment and the high cost of analysis, it has not been routinely used for determination of drug concentration in GCF samples.

Conclusion

The collection and quantitative determination of drugs in GCF will continue to play a significant role for evaluation of treatment response. The developments of modern instruments will facilitate the entire process of GCF analysis at the same time leading to facilitated analysis of novel delivery systems intended for treatment of periodontal disease and related conditions.

References

Determination of fluoroquinolone antibacterial residues in milk by LC-MS/MS method

Gjylai Alija1*, Zehra Hajrullai-Musliu2, Risto Uzunov2, Drita Havziu3, Arlinda Haxhiu-Zajmi3, Edita Alili-Idrizi3, Agim Shabani3

1,3 Department of Pharmacy, Faculty of Medical Sciences, University of Tetova, St. Ilinden 1200, Tetovo, North Macedonia
2 Faculty of Veterinary Medicine, Ss. Cyril and Methodius University, Lazar Pop-Trajkov 5/7, 1000 Skopje, North Macedonia
3 Department of Chemistry, Faculty of Natural Sciences and Mathematics, University of Tetova, St. Ilinden 1200, Tetovo, North Macedonia

Introduction

Fluoroquinolones (FQs) are a group of broad-spectrum antibiotics, derived from nalidixic acid. They are widely used to treat or prevent bacterial infections in veterinary and aquatic medicine. Also they can use as growth promoters. These residues may cause bacterial resistance, allergic hypersensitivity and toxicity effect (Junza et al., 2010). Due to their side effects in public health, the European Union has established maximum residue levels (MRLs) for most antibiotics in milk and animal tissues. Various analytical methods have been reported for analysis of FQs in food producing animals including screening and confirmatory methods. LC-MS/MS method have high sensitivity and selectivity which can allow for the simultaneously separation and confirmation of many antibiotics in a single run (Alija et al., 2016; Berendsen, 2013; Uzunov et al., 2019). The aim of this study was to develop and validation of LC-MS/MS method for determination of FQ drug residues Enrofloxacin (ENR) and Ciprofloxacin (CIP) in bovine milk and analysis of real bovine milk samples for detection of these residues.

Materials and Methods

Sample collection

In this study were analyzed a total of 250 bovine milk samples, collected from Macedonian farmers during 2016-2019.

Standards, chemicals and reagents

ENR, CIP, methanol, acetonitrile, water (LC-MS grade), trichloroacetic acid (TCA), disodium hydrogen phosphate dehydrate, disodium salt of ethylene diamine tetraacetic acid, formic acid, sodium chloride, citric acid monohydrate.

Preparation of standards

Individual stock standard solutions of 1.0 mg/mL were prepared in methanol. The range of the calibration curve was from 10 to 150 ng/mL.

Sample preparation and extraction procedure

On 5 mL of milk was added 2 mL of 20% trichloroacetic acid and the samples were shaken for 5 min. After that, 20 mL of McIlvaine buffer were added and samples were centrifuged at 4000 rpm,
20 min, at +4 °C. The supernatant was immediately applied to an SPE cartridge (Oasis HLB, 3 cc, 60 mg), previously activated with 3 mL of methanol and 2 mL of water, washed with 4 mL of water, eluted with 3 mL of methanol. The samples were evaporated to dryness under stream of nitrogen at 35 °C. The dry residue was reconstituted with 500 µL of the mobile phase and filtered on a 0.22 µm microfilter. Into LCMS/MS system injected 10 µL.

Chromatographic conditions

The compounds were separated at 40 °C using Kinetex®C18 column (1.7 µm, 50x2.1 mm). The flow rate was 0.4 mL/min and total run time was 13 min. Mobile phase A was water with 0.1% formic and mobile phase B was acetonitril with 0.1% formic acid.

MS/MS conditions

The MS/MS measurements were performed with triple quadrupole mass detector. For identification and quantification of the residues were used positive electrospray ionization (ESI+) mode and the ions were monitored in the multiple reaction monitoring (MRM) mode.

Method validation

Validation of the method was performed according to Commission Decision 2002/657/EC. Linearity, decision limit (CCα), detection capability (CCβ), accuracy and precision was determined.

Results and discussion

Each individual standard with a concentration of 10 µg/mL were directly infused into the MS/MS detector for determination of precursor and product ions. Also, fragmentary voltage and collision energy for each standard were optimized. Chromatographic separation providing satisfactory resolution with retention times between 3.43 min. (CIP) and 4.75 min (ENR). Na2EDTA-Mcllvain buffer and TCA solvents were found to be sufficiently for protein precipitation, which provided acceptable recoveries.

The linear regression analysis showed good correlation with R² from 0.9817 for ENR and 0.9946 for CIP. The CCβ values ranged from 120.51 ng/mL for ENR to 121.71 ng/mL for CIP, while the CCα values ranged from 110.58 ng/mL for ENR to 115.89 ng/mL for CIP. For determination of accuracy and precision (repeatability and reproducibility) of the method a blank milk samples were fortified with a mixture of FQs standards at three concentration levels (0.5 x MRL, 1x MRL and 1.5x MRL). The MRL for ENR and CIP is 100 ng/mL. The average recoveries for all three concentration levels varied from 88% to 109%. Precision was evaluated by CV, % (coefficients of variation). The CV for repeatability ranged from 5.23 to 20.38%, while the CV for reproducibility ranged from 4.11% to 20.80%. In the milk samples included in this study the residues of CIP and ENR above the MRL values weren’t detected.

Conclusion

The LC-MS/MS method has been developed and successfully validated according to the EU requirements to determination of fluoroquinolone residues in milk. The method can be used in routine analyses of milk samples.

References

Evaluation of biomarker activity in gingival crevicular fluid during short-term orthodontic treatment: comparison between mesial and distal sites

Angela Tasevska¹*, Liljana Anastasova², Rumenka Petkovska², Natasa Toseska-Spasova¹, Mirjana Popovska³

¹Department of Orthodontics, Faculty of Dentistry, Ss. Cyril and Methodius University, 1000 Skopje, Republic of North Macedonia
²Institute of Applied Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy, Ss. Cyril and Methodius University, 1000 Skopje, Republic of North Macedonia
³Department of Oral Pathology and Periodontology, Faculty of Dentistry, Ss. Cyril and Methodius University, 1000 Skopje, Republic of North Macedonia

Introduction

During orthodontic tooth movement, periodontal tissues respond rapidly to mechanical stress with consequent metabolic changes that allow tooth movement. This process triggers a cascade biological process involving acute inflammatory response in periodontal ligament and consequently alveolar bone remodeling (Dudic et al., 2006; Kumar et al., 2019). The extent of changes during tooth movement can be monitored by performing analysis of biomarkers in gingival crevicular fluid (GCF), such as the enzymes alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) (Kapoor et al., 2019; Kumar et al., 2019; Perinetti et al., 2002; Perinetti et al., 2003; Serra et al., 2003). The aim of our study was to assess GCF activity of ALP, AST and LDH from mesial and distal sides of the teeth during the short-term orthodontic treatment. Comparisons between distal and mesial sites were made to evaluate whether application of orthodontic force causes differences in the activity of the above mentioned biomarkers in GCF.

Materials and methods

Twenty orthodontic patients (aged 13-28) enrolled in the study underwent bilateral extraction of the first maxillary premolars as part of the orthodontic therapy. Distalization force using elastic chain was applied on one of the maxillary canines representing the test canine (TC) whereas contralateral canine was affected only by the passive force from the stainless steel wire and represented the control canine (CC). Informed consent was obtained from all patients and study protocol was approved by the Ethics Committee of the Faculty of Dentistry. GCF samples were collected before and 7, 14 and 21 days after application of orthodontic force using paper strips (Whatmann, 2x5 mm) from the distal (compression) and the mesial (tension) sides of both canines using the method proposed by Koss et al. (2009). After collection, they were placed in eppendorf tubes, 250 µL of PBS pH=7.4 (phosphate buffer saline) were added and kept at -20°C until analysis. ALP, AST and LDH activity was determined using commercially available enzyme kits, according to manufacturer’s instructions. The statistical analysis was performed using SPSS 20 for...
Windows (SPSS, Chicago, IL, USA). The Wilcoxon signed rank test was employed to evaluate significant differences (at 5% level ($P \leq 0.05$)) between mesial and distal sites, at each time point. Mann Whitney U test was used for independent sample analysis.

Results and discussion

ALP, AST and LDH activity in GCF measured at the CC showed no significant changes during treatment as a result of lack of orthodontic force. The low enzyme activity observed in every sampling point shows insignificant bone resorption and bone deposition in both sites of CC. Before application of orthodontic force, there were no significant differences in the ALP, AST and LDH activity between the distal and mesial measurement sites of the TC. The mean values for ALP activity on the mesial sites were lower the ALP activity on the distal sites. Significant differences in the mean ALP activity were observed at all occasions after the application of orthodontic force when compared to baseline. Thus, statistically significant differences in GCF ALP activity were seen from day 7 until the end of the treatment with peak enzyme activity measured 14 days after orthodontic force application. However, the comparison of ALP activity between distal and mesial sites revealed no significant differences. These findings indicate that there is an evident ongoing process of bone deposition on both mesial and distal sites of the distalized canine. Mean values for AST activity in GCF were slightly higher on the distal side compared to the mesial side, but the differences were not statistically significant. Peak AST activity was determined on 21 day after beginning of the treatment. This is probably due to the greater bone resorption process that occurs on the compression site. Peak LDH activity was observed on day 21 of the treatment. The analysis of LDH activity revealed higher enzyme activity on the mesial than the distal site of the tested canine. This finding implies on greater bone resorption that occurs on the compression site of the distalized canine due to distalization force. The comparison of AST and LDH activity on the compression sites revealed no significant differences during short-term orthodontic treatment.

Conclusion

The application of orthodontic force resulted in changes in the activity of ALP, AST and LDH in GCF. The results suggest that these biomarkers may reflect a part of the biological activity in the periodontium during early phases of orthodontic treatment and therefore be used to prevent and stop adverse effects from the orthodontic treatment.

References


Quantification using GC/ECD: challenges and pitfalls

Ana Poceva Panovska*, Jelena Acevska, Gabriela Petrovska Dimitrievska, Katerina Brezovska, Natalija Nakov, Zoran Kavrakovski

Faculty of Pharmacy, Ss. Cyril and Methodius University, Majka Tereza 47, 1000 Skopje, North Macedonia

Background

Gas chromatography (GC) combined with electron capture detection (ECD) is commonly used techniques for analyzing organo-halogenated molecules which offers high selectivity and sensitivities at concentration ranges of parts-per-billion (ppb) or even parts-per-trillion (ppt). Comparing to the universal flame ionization detector (FID), an ECD can be 10-10^3 times more sensitive depending on the analyte tested. Despite the advantages, ECD is often described as a detector with limited dynamic linear range with linearity ranging between 10^3-10^4 (Rood, 2007). Problems with the linearity are quite essential for quantifying an analyte because they define the range of the method within which the results are obtained accurately and precisely (Booij et al., 1998).

The aim of this review is to list some of possible reasons that might be involved in the non-linear response of the ECD that could often be source of ambiguous quantification, as well to address some of practical approaches to solve that.

ECD related non-linearity

The first point to inspect shouldn’t be the detector itself, but the samples, especially if the onset of the non-linearity problem is unexpected. Inaccuracies in preparation, possible degradation of the substances, and evaporation of solvent and consequently variations in concentration are few of potential causes of non-linearity.

Any changes in the injection process also can alter the amount of sample entering the column and further significantly affect the linear response in the experiment. Inconsistent manual injection gives difference in the applied amount that usually leads to loss of repeatability and worsening of linear response. Adsorption of the analytes by the column or injector liner, may contribute to poor linear response since there is a reduction in the amount of compound that reaches the detector. In general, a compound at a lower concentration is more affected than the same compound at a higher concentration, and this often results in nonlinear calibration curves.

The detector is another potential source of nonlinear calibration curves. ECD is an ionization detector and its response is based upon the ability of molecules with certain functional groups to capture electrons generated by the radioactive source. The non-linearity characteristics of the ECD are often due to the physics of the response mechanism, since it is a “subtractive” detector. It means that maximum signal is obtained with no compounds in the detector by a steady background current that is formed by ions using the ionization source (63Ni). The electro-negative samples going through the detector, reduce the signal (by capturing electrons) versus adding signal in most other detectors, explaining the poor (linear) dynamic range of the ECD. (McNair and Miller, 2009)

Decrease in the linear range is one of the first signs of a contaminated ECD cell with high concentration of analyte. Due to contamination background current in the detector reduces, but the current reduction per analyte molecule remains

* anpo@ff.ukim.edu.mk
constant which leads to non-linear response. Contaminated detectors usually give rise to negative ‘dip’ after each peak.

Another issue concerning the linearity is that ECD is a concentration type of detector. It measures concentration of the analyte in the carrier gas compared to other detectors (FID) that directly measure the absolute amount of analyte irrespective of the volume of the carrier gas. Due to this characteristic of ECD, quantitative data acquired at different flow rates will be affected. Therefore operation at constant flow may be necessary for quantitative analysis in programmed temperature GC. Additionally make-up gas should also be used. Lower rates of make-up gas will increase the sensitivity but it also increases the non-linearity. (McNair and Miller, 2009).

Practical approach in solving the non-linearity

When developing GC-ECD method for quantification purposes in laboratory few practical approaches should be consider and tested in order to check and solve potential non-linearity.

Check the linearity? Is the concentration of the analytes within the linearity range of the ECD?

Linear range and its end points have to be determined experimentally as they are often different for various compounds. If multiple point calibration curves are not generated, the loss of linear range may not be noticed until the errors in the quantitative results become quite severe. One of the approaches to alter the column/detector loadings is to increase the split flow but dilution of the too concentrated samples and re-injection is sometimes necessary.

Are there obvious reasons for the nonlinear curve?

Check sample concentration (they may fall outside the linear range of an ECD), injection technique, adjust the split flow, column flow and make-up flow. Look at the results of calibrations because sometimes the problem might be poor repeatability, not necessarily poor linearity. To avoid sample mistakes preparing additional set of samples or using another GC are the easiest methods to determine accountably of the prepared samples.

Check for possible coelution analytes?

In multicomponent analysis co-elution of components may contribute to non-linearity. Therefore the selectivity of the column for the compounds of interest must be checked. Column substitute might be a solution.

Can the dependence between the concentration and the response be explained by another mathematical model?

The log transformation can decrease the variability of data and make data conform more closely to the normal distribution. However, the results of standard statistical tests performed on log-transformed data are often not relevant for the original, non-transformed data, so this approach must be applied very cautiously. Instead newer analytic methods that are not dependent on the distribution the data (Feng et al., 2014) should be used.

Conclusion

Lack of linearity is often source of ambiguous quantification using ECD. There are some possible experimental hints that need to be determined in order for the linearity to be achieved. If the linearity remains questionable, the method should only be intended for semi-quantitative determination of the analyte concentration. Where possible, an alternative detector with broader linearity range and suitable sensitivity could be used.

References


Development and validation of a RP-HPLC method for simultaneous determination of terbutaline sulfate, guaifenesin, bromhexine hydrochloride and sodium benzoate in a syrup formulation

Marjan Piponski*, Tanja Bakovska Stoimenova, Kristina Grnacharoska, Martina Miloshevska, Irena Slaveska Spirevska, Emilija Pockova, Elena Petrovska, Marjan Velkovski

Replek Farm Ltd., Kozle 188, 1000 Skopje, N. Macedonia

Introduction

Terbutaline sulfate is a selective beta-2 adrenergic agonist used as a bronchodilator, used to treat wheezing and shortness of breath from lung problems (e.g. asthma, chronic obstructive pulmonary disease, bronchitis and emphysema).

Bromhexine hydrochloride is mucolytic, which helps clear chest congestion, thus contributes to a secretomotoric effect. It also has antioxidant properties.

Guaifenesin is an expectorant, working by thinning and loosening mucus in the airways, cleaning congestion, and making breathing easier. It is used to treat coughs and congestion caused by the common cold, bronchitis, and other breathing illnesses (McCrory et al., 2013).

Sodium benzoate is used as a preservative in the syrup formulation.

There are a number of methods available for determination of terbutaline sulfate, bromhexine hydrochloride and guaifenesin individually (Ph. Eur., BP, USP), but only a few methods for simultaneous determination of a combination of them in a syrup formulation.

The aim of our work was to develop and validate a simple and rapid reversed-phase high performance liquid chromatography (RP-HPLC) method for simultaneous estimation of the active substances, terbutaline sulfate, guaifenesin, bromhexine hydrochloride and the preservative sodium benzoate, in a cough syrup formulation.

Materials and methods

The reagents that have been used are: ammonium dihydrogen phosphate (NH₄H₂PO₄) and 85% o-phosphoric acid (H₃PO₄) purchased from Sigma Aldrich, USA, methanol and acetonitrile procured from Merck, Darmstadt, Germany, and the demineralized water was “in house” prepared with conductivity of 0.05 µS/cm. The terbutaline sulfate reference standard, bromhexine HCl CRM, guaifenesin CRM and sodium benzoate analytical standard, were purchased from Sigma-Aldrich, USA, and the syrup formulation was obtained from Replek Farm Ltd., Skopje, N. Macedonia. The syringe filters Nylon and RC, 0.45 µm, were purchased from Agilent Technologies (USA).

* piponski99@gmail.com
Instruments that have been used are: UPLC Shimadzu Nexera XR system with LPG quaternary pump with degasser, autosampler, controller and PDA detector and column oven, controlled by Lab Solutions software, version 5.97; analytical balance Mettler Toledo AG285; pH-metter Metrohm 827 pH Lab; and IKA orbital shaker KS 260 basic.

The separation was accomplished using Inertsil RP8 250 mm × 4.6 mm, 5 µm column from GL Sciences, Tokyo, Japan.

Results and discussion

The chromatographic separation of all three active substances and the preservative (all with significant differences in their concentrations, polarities, solubility, UV absorbing spectra and molar absorption coefficients) was carried out on a GL Sciences Inertsil RP8 250 mm × 4.6 mm, 5 µm column, under isocratic conditions, with mobile phase consisting of 20% v/v methanol, 20% v/v acetonitrile and 60% v/v 20 mM NH₄H₂PO₄ pH 2.5, with flow rate was 1.2 mL/min, detection wavelength at 220 nm, column temperature of 30 °C and injection volume of 5 µL.

The isocratic elution of the analytes was achieved in 12 min, with retention time of terbutaline sulfate, guaifenesin, sodium benzoate and bromhexine HCl on 2.3, 4.6, 7.6 and 9.7 minutes, respectively. All four chromatographic peaks are well separated between each other, to the baseline. The obtained values for number of theoretical chromatographic plates for terbutaline sulfate, guaifenesin, sodium benzoate and bromhexine HCl, were 4025, 10201, 12641 and 10151, respectively.

The established method was validated according to the International Conference on Harmonization (ICH) Q2(R1) guideline for validation of analytical procedures. During selectivity testing, no interference from the formulation excipients was observed. The linearity of the method was proved in five concentration levels, for each substance of interest and the following results were obtained by regression analysis: correlation coefficient > 0.9990 and relative standard deviation of the response factors for each concentration level < 2%, in all cases. The precision of the system and of the method were also evaluated and the obtained relative standard deviation of the responses was less or equal to 2%, in both cases, for each substance. Accuracy of the method was studied by recovery investigation. The obtained recovery values were within the range of 100±2%, for each substance. The robustness testing of the method, showed that the obtained results are not adversely affected by small variations in method parameter.

Conclusion

The developed RP-HPLC method enables simultaneous, fast and accurate determination of the three active substances, terbutaline sulfate, guaifenesin and bromhexine HCl, and the preservative, sodium benzoate, all with different physico-chemical properties, in syrup formulation. The method was validated and proved as suitable for its intended use. The proposed method, using simple sample preparation and low cost reagents, provides reproducible quantification of all substances of interest and can be successfully used for routine analysis or cough syrups containing this combination of active substances.

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A fast and simple HPLC method for determination of mesalazine impurities A and C in raw material and finished pharmaceutical products

Marjan Piponski*, Tanja Bakovska Stoimenova, Irena Slaveska Spirevska, Stefan Stefov, Elena Lazarevska Todevska, Marina Topkoska, Gordana Trendovska Serafimovska

Replek Farm Ltd., Kozle 188, 1000 Skopje, N. Macedonia

Introduction

Mesalazine, also known as mesalamine or 5-aminosalicylic acid (5-ASA), is a medication used in the management of inflammatory bowel disease, including ulcerative colitis and Crohn's disease. It is generally used for treatment of mild to moderately severe conditions, especially for maintenance of remission (Iacucci et al., 2010).

According to the monograph of mesalazine in the current edition of the European pharmacopoeia (Ph. Eur), this active substance has thirteen specified impurities and a few other detectable impurities, which can be determined by three analytical procedures using different chromatographic conditions. The monograph of mesalazine in Ph. Eur (as well as in the British pharmacopoeia-BP) prescribes one HPLC method for determination of impurities A and C, another HPLC method for determination of impurity K, and a third HPLC method for analysis of the parameter related substances (determination of the other ten specified impurities, as well as unspecified impurities). The test for determination of impurities A (4-aminophenol) and C (2-aminophenol) prescribes gradient elution HPLC method, with a run time of 29 minutes, using acid mobile phase composed of 1.0 g/L phosphoric acid R and 2.2 g/L perchloric acid R in water, and 1 g/L phosphoric acid R and 1.7 g/L perchloric acid R in acetonitrile, with flow rate 1.0 mL/min, use of acidic pH resistant end-capped Nucleodur C18 HPLC column 250 mm × 4.6 mm, 3 µm, working at backpressure of about 300 bars, achieving separation potency of more than 15000 NTP (number of theoretical plates) from the available theoretically calculated 42000 NTP. The mesalazine monograph of in the United States pharmacopoeia (USP) prescribes gas chromatography (GC) method for determination of these two impurities.

The aim of this study was development of a fast and simple, isocratic chromatographic method for determination of mesalazine impurities A and C.

Materials and methods

The following reagents were used: methanol, 85% o-phosphoric acid and 70-72% perchloric acid, purchased from Merck, Darmstadt, Germany; potassium hexafluorophosphate (KPF6), ≥99% purchased from Sigma-Aldrich, USA; while the deionized water was “in house” product prepared with conductivity of 0.05 µS/cm.

The following instruments were used: analytical balance Mettler Toledo AG285, pH–metter Metrohm 827 pH Lab and IKA orbital shaker KS 260 basic.
The RC (regenerated cellulose) 0.45 µm syringe filters were purchased from Agilent Technologies (USA).

Two HPLC systems were used: Dionex Ultimate 3000 UHPLC system controlled by Chromleon software, version 6.80, composed of quaternary LPG pump, autosampler, column compartment and four channel UV-Vis detector; and Shimadzu Nexera XR UPLC system with LPG quaternary pump with degasser, autosempler, PDA detector, column oven and controller, controlled by Lab Solutions software, version 5.97. The HPLC column, Discovery C18 150 mm × 4.6 mm, 5 µm, was purchased from Sigma-Aldrich, USA.

The active substance mesalasine was obtained from Replek Farm Ltd., and the impurities, mesalazine impurity A CRS and mesalazine impurity C CRS were purchased from EDQM. All sample and standard solutions were prepared in accordance with the method for testing of impurities A and C from Ph. Eur. monograph for mesalazine active substance.

Results and discussion

We developed a fast and simple isocratic chromatographic method using a different concept of mobile phase composition, based on the recognized chaotropic characteristics of potassium hexafluorophosphate (KPF₆) dissolved in acidic medium, enabling increased column retention of molecules containing nitrogen atom in their structure (Kazakevich & LoBrutto, 2007).

The mobile phase consisted of 2% v/v acetonitrile and 98% v/v buffer composed 0.05% v/v o-phosporic acid with added 0.3% w/v KPF₆. The developed method uses HPLC column Discovery C18 150 mm × 4.6 mm, 5 µm, UV detection at 220 nm, mobile phase flow rate of 1.0 mL/min and column oven temperature set at 35 °C. These chromatographic conditions generated different appearance of the chromatogram regarding the retention times of mesalazine and its impurities A and C. Using the method prescribed in the Ph. Eur. monograph, elution order of the substances of interest is the following: impurity A with RRT ~0.5, impurity C with RRT ~0.9 and mesalazine. Using the new developed method, the obtained elution order is different: impurity A with RRT ~0.8, mesalazine with RT ~3.4 minutes and impurity C with RRT ~1.4. The separation between all substances of interest was satisfactory and complete.

Analysis of mesalazine active substance was performed using the method from the Ph. Eur. monograph for mesalazine and the new developed method. The obtained results for impurities A and C of mesalazine were comparable for both methods, with relative difference of each result for every specified impurity lower than 2%.

Conclusion

The advantages of the newly developed isocratic HPLC method for determination of mesalazine impurity A and C are the following:

- The use of isocratic elution enables shorter run time of about 6 minutes, versus the gradient elution described in Ph. Eur. and BP monographs for mesalazine, requiring longer run time of about 29 minutes;
- The use of shorter RP C18 column with 5 µm particles resulted in separation power of about 10000 NTP (available theoretically calculated is 15000 NTP), instead of longer, special acid-resistant C18 column with 3 µm particles, enabling shorter time for column equilibration and analysis;
- Lower working backpressure using the new developed method, up to 85 bars, versus ~300 bars using the HPLC column and chromatographic conditions prescribed in the Pharmacopoeia.

References


Safety aspects of products, food supplements, intended for weight loss treatment

Blagica Samarova Stoëv*, Dafinka Damcevska, Silvija Saveska, Tatjana Bogovska, Nada Stojanoska, Mena I. Zdravkovska, Hristina Babunovska

1Alkaloid AD-Skopje, Pharmaceutical, Chemical and Cosmetics Industry, blvd.Aleksandar Makedonski 12, 1000 Skopje, North Macedonia

Introduction

Nutrition supplements are food products that supplement the normal diet and are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, released in dosage form as capsules, lozenges, tablets, sachets with powder substance, liquid vials and other similar forms designed to be used in small quantifiable quantities. This product group includes vitamins, minerals, amino acids, enzymes, pre and pro biotics, essential fatty acids, herbs and extracts, mushrooms, algae, various bioactive substances.

The pharmacodynamic effects of the supplements are due to the presence of certain active ingredients in them. Efficacy is based on centuries of experience since there are few well-controlled, randomized, clinical trials.

Overview of the regulatory framework on the safety aspects of products and food supplements intended for weight loss treatment

The increasing number, as well as the consumption of nutrients and other ingredients that can be presented as dietary supplements and the different national rules for their marketing have led to the adoption of Directive 2002/46 / EC within the EU. The Directive represents:
- Harmonized regulatory framework for national law for EU Member States;
- Contains a harmonized list of vitamins and minerals that can be added for nutritional purposes in food supplements; Annex I - types of vitamins and minerals; Annex II - Form and purity of substances;
- Harmonized rules for labeling supplements whether it has a health or nutritional label.

Directive 96/8 / EC regulates food intended for energy-restrictive diets for weight loss. According to this directive, our "Rulebook on food safety intended for use in restricted-value diets to reduce weight" has been developed. Rulebook foods intended to be used in diets with a limited energy value for weight loss purposes are foods with a specific composition which, if used in accordance with the instructions given by the manufacturer in whole or in part, replaces overall daily meals.

Foods with limited energy value are divided into two categories:
1. Food that is presented as a complete replacement of daily meals;
2. Food that is presented as a substitute for one or more daily meals.

* bsamarova@alkaloid.com.mk
EFSA represents EU pillar on risk assessment and food safety on the territory of European Union.

The DSHEA is a FDA-law on US territory that regulates dietary supplements since 1994. This law requires any supplement to be marked as such; provide reasonable evidence (expectations) of safety, but does not require the manufacturer to have FDA-approved product safety prior to being placed on the market. Once the dietary supplement is released to the market, the FDA has the responsibility to monitor product safety through mandatory reporting of adverse events by manufacturers, consumers and health professionals.

**Challenges and perspectives**

Weight loss supplements contain many ingredients such as minerals, vitamins, amino acids, enzymes, fiber, herbs in various amounts and in many combinations in the form of capsules, tablets, liquids or powders. The main ingredients of weight loss supplements are: African Mango, Beta Glucani, Bitter orange, Caffein, Calcium, Capsaicin, Carnitin, Chitosan, Chromium, Coleus forskohlii, Conjugated linoleic acid, Fucoxanthin, Gastrovia cambogia, Glucomanan, Green coffee bean extract, Green tea, Guar gum, Hoodia, Probiotics, Pyruvate, Rasberry ketone, Vitamin D, White kidney bean, Yohimbe.

Most products contain more than one ingredient, and the ingredients can function differently if combined. Therefore, it is actually complicated to discover the safety of these preparations during use for their intended purpose, weight loss. There can be safety evidence for only one ingredient in the finished product, and no evidence of the efficacy and safety of others is available. In addition, the doses and the amount of active ingredients vary greatly among weight loss supplements, and the composition of the product is not always fully described in published clinical trials.

Manufacturers of weight loss supplements rarely conduct clinical studies in humans to find out the efficacy and safety of the product. Even when clinical trials are done, they usually involve a small number of subjects using the product for a short period of only a few weeks or months. Studies may also use different and sometimes inappropriate techniques to evaluate the efficacy and safety of a preparation.

**Conclusion**

Concerning the safety of supplements, there are limited data, without preclinical studies, an insufficiently known safety profile, the occurrence of drug interactions that emphasize or diminish its effect, the effect of absorption, metabolism or excretion of the drug or the occurrence of adverse drug reactions. Allergic reactions, GIT disorders, liver, kidney, CNS disorders, sedation, depression, etc.

Natural, doesn't always mean safe and secure.

**References**

**Herbal food supplements safety and future regulation challenges**

Marjan Dzeparoski

*Bionika Pharmaceuticals, Skupi 57, 1000 Skopje, North Macedonia*

**Introduction**

A general framework for safety assessment is proposed by the EFSAs Scientific Committee, in which botanicals or botanical preparations for which an adequate body of knowledge exists could benefit from a “presumption of safety” without any need for further testing, based on long history of use without reported adverse effects and without significant larger exposition. Botanicals and botanical preparations for which a presumption of safety is not possible based on available knowledge would be subject to a more extensive safety assessment, requiring additional data to be provided. For these preparations with a potential to contain toxic, addictive, psychotropic or other substances that may be of concern (given in the Compendium), presumption of safety can be applied only if there is convincing evidence that these undesirable substances in the specific plant parts or preparations are either absent in the source material, or significantly reduced if not excluded, or inactivated during processing (EFSA Journal, 2009).

**Discussion on regulatory challenges**

Herbs and their preparations obtained from plants, algae, fungi or lichens are widespread in the EU in the form of food supplements. They can be purchased at pharmacies, supermarkets, and specialty stores or online. Although most of these products have a long history of use in Europe, there are some concerns in terms of safety and quality. Contamination (chemical and microbiological) is a problem, associated for example with herbal products from Asia. Food supplements use is associated with 23000 emergency department visit and 2000 hospitalizations in the United States each year (Geller et al., 2015). Adulteration with synthetic drugs is prevalent in, although not limited to food supplements intended to weight-loss, sport performance and erectile and libido dysfunction. Cases of death in Europe and the United States consuming products contaminated with heavy metals, synthetic drugs and other undesirable substances are reported.

Although absolute safety does not exist, information about traditional use is an important element in the assessment of the safety of traditionally used botanicals and their preparations. History of use should, as a minimum, cover use during one generation - 25 years. It has advantages for manufacturers and regulators. When botanicals are used in equivalent forms and under comparable conditions for use, as described in traditional information and after analyzing all existing data, it ensures that the botanicals and their preparations used in the food supplements will not endanger the health of consumers. In the absence of reports or other safety-relevant information, it may be used to avoid the need for further toxicological tests (Anton et al., 2012). But this can obviously not exclude the
emergence of new security issues that have not yet been identified. An important aspect of the food law is the so-called obligation to notify, arising from Article 19. This routine surveillance request, combined with the RASFF, links all the European security authorities of the Member States and constitutes a powerful tool for identifying unexpected untoward effects of specific herbal food supplements in the early stage and taking appropriate actions like recalls (Coppens et al., 2006). RASFF Consumer Portal, the consumer friendly internet tool was launched in June 2004. Through RASSF has been registered occurrence of yohimbine, vinpocetine, evodiamine, huperzine A, vincamine, vinburnine, arecoline, higenamine, Evodia ruteacarpa, Huperzia serrata and other unauthorized substances in the food supplements. Post-marketing surveillance is therefore an essential tool, organized by some member states in a systematic way.

In France, for example, ANSES implemented the "Nutrivigilance" system in 2010 and health professionals can report an adverse health reaction directly online or private individuals/patients can do this through their doctor or pharmacist, or directly through the French Ministry of Health’s adverse event reporting portal. In the framework of the Act of 13 October 2014 on the future of agriculture, food and forests, ANSES has been entrusted with setting up a phytopharmacovigilance scheme.

In recent years there has been an increase in reported adverse events from the use of food supplements. This is also because of the mandatory reporting to FDA of serious adverse events (events that require medical intervention to prevent death, hospitalization or birth defect) for dietary supplements from 22 December 2007 by manufacturers, packers and distributors.

**Conclusion**

Since the early history of humans and up until now botanicals have been widely used. Popularity of food supplements is increasing all over the world, which is helped with the perception that herbal food supplements are natural and therefore safe. The market of herbal food supplements is expected to grow even further in the future as disease prevention, healthier living, modern habits, longer quality lifespan are a top concern of many people. Contamination (chemical and microbiological) is a problem, associated for example with herbal products from Asia. Cases of death in Europe and the United States consuming products contaminated with heavy metals, synthetic drugs and other undesirable substances are reported. Food supplements can and provoke adverse events. They can have pharmacological effects and therefore can have serious adverse events. Traditional use is a key source of information to demonstrate the safety of many botanicals and their preparations. Safety of food supplements that for is of utmost importance. Despite RASFF in Europe, post-marketing surveillance is an essential tool organized by some states in a systematic manner to protect consumers. In other countries suspected adverse events from food supplements can only be reported as part of the concomitant therapy with reports for the medicinal products or medical devices, where probable cause and effect relationship can be established. In France ANSES implemented the Nutrivigilance system in 2010. In USA, in recent years there has been an increase in reported adverse events from the use of food supplements. This should be followed also by other states together with regulation harmonization, which will mitigate their risks and make the use of herbal/food supplements with nutrivigilance much safer.

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Drug-device combinations and Article 117: more questions than answers?

Elena Ivanovska¹*, Jelena Lazova¹, Suzana Trajkovic Jolevska², Jasmina Tonic Ribarska², Nada Popstefanova¹, Marija Davcheva Jovanoska¹

¹Pharmaceutical Chemical Cosmetic Industry ALKALOID AD Skopje, Blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia
²Faculty of Pharmacy, Ss Cyril and Methodius University, Str. Majka Tereza 47, 1000 Skopje, Republic of North Macedonia

Introduction

While the medical device industry is dealing with the medical device regulation (MDR) and changes it brings along, the drug-device combination products (DDCs) are facing an additional uncertainty: the provisions of Article 117. In lay terms this article basically says that, as from May 2020, all new DDCs should be in compliance not only with the legislation requirements for medicinal products, but as well as with the requirements of the new regulation for medical devices.

Regulatory context

Article 117 of the MDR 2017/745 introduces a new requirement for medicines with an integral device (CoE, 2017). The marketing authorisation application (MAA) should include results of the assessment of the conformity of the device part with the relevant general safety and performance requirements (GSPRs) set out in Annex I in a form of a CE certificate or declaration of conformity for the device or, if it is not CE marked but would need to be certified if marketed separately, the applicant must include an opinion from a Notified Body (NB) on the conformity of the device (EMA, 2020).

Defining drug-device combination products

The MDR does not provide clear definition of drug-device combination products, but the European Medicines Agency (EMA) has managed to provide some description and classification in the draft guideline that they have issued. According to this guideline terms ‘integral’ and ‘non-integral’ are used to describe the DDCs (EMA, 2019a).

Guidance and open questions

Earlier in 2019, EMA published a Q&A guide, which addresses several key issues, but still there are points that remain to be further resolved, such as:

- Involvement of NB designated to carry out the conformity assessment procedure for the particular medical device type(s) where certification is sought.
- Limited number of NBs are certified under the MDR, with limited scope; affecting not only the possibility of finding the proper NB for performing the assessment but also the timeframes for submission of MAA.
- MAAs for these products will be jointly assessed by competent authorities and NBs.
- What happens if the reviewers disagree on one or more of the overlapping combination product attributes or risks (EMA, 2019b; Pillar, 2019).

* eivanovska@alkaloid.com.mk
If the device is governed by the medicinal products legislation, then MDR obligations related to labelling and UDI are not required and should not be applied to the package of the combination product.
- This exclusion may not be in the interest of safe products for patients and other users since labelling requirements for medical devices differ from the requirements for medicinal products. Confirmation of compliance with MDR is based on assessment of Annex I solely but it does not include evaluation of device’s clinical data.
- Clinical data on the device may be very useful for performing clinical studies on the DDC (EMA, 2019a; Steffen, 2018).

What about the device point of view?

All of the EMA guidance on assuring conformity of the device is mainly focused on which documents should be provided, who is to provide them and where should they be placed on the CTD, but there are more important issues as how to assure the compliance of the device with Annex I of the MDR.

The current situation is rather confusing when looked also from medical device manufacturer point of view. One example is the use of harmonised standards, which are essential for demonstrating compliance with GSPRs. When we look closer, it becomes apparent that there is a significant amount of technical standards that demonstrate compliance with the GSPRs but that are not harmonized. Also, the recent harmonization was done under the MDD without consideration for the MDR. In order to get the necessary harmonization started, the European Commission has to issue respective standardization requests as well as conduct a defined review process considering the GSPRs. While this overall process is time consuming and requires involvement of several parties it is expected that many standards will not be harmonized under the MDR in time.

Furthermore, no Common Specifications have been published that need to be considered in the context of a single integral DDC.

The applicability of the GSPRs to a DDC is partially hard to break down to the device part only. In fact, the interaction of device and drug product needs to be considered for many requirements such as to test functionality characteristics, or stability testing or obtaining clinical data (Schommer, 2019).

Conclusion

For DDCs manufacturers the continuous vigilance of the everyday updates and changes in the legislation would now play a key role in providing quality MAA documentation in timely manner.

A lot of work still needs to be finished; a lot of questions will probably be answered throughout the processes. But in any case it is certain that all involved entities are putting all their efforts into providing the best possible solutions for ensuring safe products for patients and users, which, after all, is the ultimate purpose of all legislation changes.

References


Good Distribution Practice in preserving the integrity and safety of the supply chain of pharmaceuticals

Filip Cvetanovski¹*, Nikola Kocev¹, Jasmina Tonic-Ribarska², Suzana Trajkovic-Jolevska²

¹Alkaloid AD Skopje, Blvd. Aleksandar Makedonski, 12, 1000, Skopje, Republic of North Macedonia
²Faculty of Pharmacy, UKIM, Mother Teresa Str., 47, 1000 Skopje, Republic of North Macedonia

Introduction

The Good Distribution Practice (GDP) represents a collection of standardized, routine methods of work, which ensure that the characteristics defining the quality, safety and efficacy of the pharmaceutical products remain intact from the beginning to the end of their stay in the pharmaceutical supply chain, (European Commission, 2013). According to these practices, the pharmaceutical products should be stored according to the prescribed storage conditions, especially during the process of transportation. Since the manufacturers of the pharmaceutical products that also distribute their assortment are inspected and authorized in the Good Manufacturing Practice (GMP), they are exempted from the obligation of acquiring separate authorization for distribution. Every other legal entity has to be authorized to do so. The principles laid in the GDP expand outside of the pharmaceutical products’ distribution and include the procurement, storage and transport of active substances and other ingredients used in the manufacturing process. The global pharmaceutical chain is an immensely complicated organization.

However, its all-encompassing nature can either provide many opportunities for greater efficiency or render it susceptible for emergence of weak points that can ease the entrance of illicit products.

Probably the greatest risk for the legal distribution chain are the falsified pharmaceutical products.

These products pose a threat for the general well-being, health and the life of the unfortunate patients that are exposed to them. There are estimations that one tenth of the pharmaceutical supply chain in the developing countries consists of falsified medicines (WHO, 2018).

Good Distribution Practice

Protecting the distribution chain encompasses several important points. Firstly, it is important to draw up maps according to which the distribution shall be completed (introduction to the routes and the conditions of the transportation process).

According to this information, an assessment of the risks should be made. The products’ and the distribution chain’s control is dependent on the risk analysis, which is a constantly changing subject, due to the non-stop control and monitoring of the products’ safety (Ulrich, 2017). It can generally be considered that the pharmaceutical products coming directly from the production site to the pharmacy or the authority dispensing them to the patients are legitimate and not falsified. The risks of falsified products entering the supply chain emerge when the product changes distributors several times.

* fcvetanovski@alkaloid.com.mk
Additionally, many products are re-packed, re-labelled etc. Even their storage can be inconsistent in terms of location. Another problem is posed by the reverse-distribution (returned products). In all these points, the products can be stolen, altered and changed, which not only damages the companies financially, but also is a major public health threat (IOM, 2013). Essentially the protection of the supply chain can be facilitated by following the rules and propositions laid out in the regional guidelines on the matter, such as the Guideline on GDP of 2013 issued by the European Commission, the WHO Guideline on Good Storage and Distribution Practices (WHO, 2019) and the USP chapter 1079 on Good Storage and Distribution Practices for Drug Products (USP, 2019). These guidelines propose several points of consideration such as the importance of implementing an all-encompassing, comprehensive quality management system, that should be drawn up as a set of interactive elements based on procedures, rules, resources and goals that are collectively established with the mission of running an organization. The obligation that the procurement, distribution, storage and transportation should be done with the characteristics laid out in the GDP; the clear definition of the management’s and other personnel’s roles and responsibilities, the practice of documenting every activity, recording and investigating every deviation from the defined procedures, are considered as the essential requirements that a fully functioning quality management system should be able to perform. Such system has to be built around the ability to correctly assess and predict risks, i.e. operating around an appropriate risk-management system (ICH, 2005).

The main postulates for managing the risks are basing the evaluation of the risks to the quality on scientific knowledge, levelling the effort, formality and documentation of the processes in equivalence with the risks and ultimately focusing on the patients. Proper and responsible management of the personnel of the company, the activities of the responsible person and the trainings should encompass every activity done within the company.

The premises and equipment must be suitable and adequate to ensure proper storage and distribution of medicinal products. The document management, the definition of the different operations (qualification of suppliers and customers, receiving products, storage etc.) should ensure that the identity of the medicinal products is not lost and that the distribution is performed according to the information on the outer packaging. The complaints, returned and recalled products, the treatment of falsified medicines, outsourced activities, self-inspections and the provisions for the brokers are points of consideration.

Conclusions

The patients’ health is undoubtedly a priority for the pharmaceutical industry. That being said the good distributional practices and their proper implementation have an immense role in sustaining the quality, safety and efficacy of the distributed pharmaceutical products by preventing incidents such as falsifying, theft, manipulation, illicit transfer and the temperature excursions - changes in the temperature during transportation.

These rules secure that the right pharmaceutical product will reach the right recipient at the right time in the right condition.

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ISO 14971:2019 - Implication to the medical devices manufacturer’s quality system with emphasis of post – production activities

Marija Davcheva Jovanoska¹*, Elizabella Karadzinska¹, Nada Popstefanova¹, Olivera Paneva¹, Suzana Trajkovic Jolevska², Jasmina Tonic Ribarska¹, Jelena Lazova¹, Elena Ivanovska¹

¹Pharmaceutical Chemical Cosmetic Industry ALKALOID AD Skopje, Blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia
²Faculty of Pharmacy, Ss Cyril and Methodius University, Str. Majka Tereza 47, 1000 Skopje, Republic of North Macedonia

Introduction

Historically, risk management has been a complex subject, with different stakeholders assigning different values on the probability and severity of harm. In medical devices, its high importance has necessitated ISO 14971 - Application of risk management for medical devices, providing a generic risk-management framework applicable to all medical devices, from design and development through production and post-production activities. In December 2019, the new updated version of the ISO 14971 was released. What does this update mean to medical devices manufacturer’s quality system? A transitional period of three years allows manufacturers to successfully implement the new requirements in their quality system.

Relationship between ISO 14971:2019 and Medical Device Regulation (MDR)

Manufacturers of medical devices have a major challenge ahead of adapting the quality management system to meet the new requirements of ISO 14971:2019 and MDR. It is a mitigating circumstance that the new ISO 14971 is better aligned with the general safety and performance requirements of the MDR (Council of Europe, 2017; ISO, 2019). While no major changes have been made to the overall process of how to conduct risk-management, manufacturers will need to spend some time examining the details associated with each change in the new standard to ensure that the quality system is completely aligned with each requirement.

More requirements for production and post-production activities

Manufacturers of medical devices should comply with requirements of ISO 14971 for production and post – production activities from ISO 14971 and the MDR. The MDR talks about a risk management process, ISO 14971 about a risk management system. Production and post – production activities include four phases, each with detailed activities to be properly implemented in the system:
1. Establish a system to collect and review information from production and post market activities.

2. Collect relevant information for the medical device (i.e., information from users, distributors, publicly available information, literature, etc.).

3. Review the information gathered in phase 2 to determine its relevance to device safety. Any previously unidentified hazards or hazardous situations, new risks, or significant changes affecting the risk need to be assessed to determine if a new benefit-risk assessment is warranted.

4. Implement actions by reviewing the risk management file to determine whether new risks need to be assessed or previous risks require reassessment. This phase also includes determining whether actions are necessary for devices already on the market and assessing the impact of previous risk management activities. Additional risk control measures may need to be implemented (ISO, 2019; Trevino, 2019; van Vroonhoven, 2019).

The policy for establishing criteria for risk acceptability and the resulting criteria must take general safety and performance requirements into account, meaning for example that risks must be reduced as far as possible as long as it does not adversely affect the benefit risk ratio. The new standard specifies requirements for production and post-production information to be considered as part of the overall risk assessment process throughout the life of the device. Both the MDR and the 3rd edition of ISO 14971 require proactive collection and evaluation of data from post-development phases that must be properly included in manufacturer’s quality system. The principles of collecting and reviewing information have not changed, but the requirements and the activities are described more elaborately and more precisely (Council of Europe, 2017; ISO, 2019; Trevino, 2019).

Conclusion

ISO 14971:2019 provides a thorough process for manufacturers to identify medical device hazards, assess risks, control risks, and monitor the effectiveness of risk controls throughout the life of a device. This new edition is aligned with the general safety and performance requirements within the new EU MDR. It is now impossible to image that a medical device would be developed and placed on the market without thorough risk assessment or without post-production monitoring.

While the existing changes are aimed at clarifying concepts and no changes have been made to the overall process to conduct risk management, manufacturers still need to consider device-specific standards. These can be used - in addition to ISO 14971 - to control specific risks associated with some unique device categories to demonstrate how risks can be reduced to acceptable levels using the data from production and post-production activities. It is anticipated that some manufacturers of medical devices will have to spend some time updating references to the previous standard in existing quality system documentation.

Risk management is a challenging process in the industry, and in our view, these changes aim to clarify and simplify requirements. The end goal should be to improve the effectiveness of the risk management process to consistently launch highly beneficial, safe and effective medical products by proper implementation of the requirements for production and post-production activities in the manufacturer’s quality system.

References


Community pharmacies in North Macedonia – legal status

Bistra Angelovska*, Elena Drakalska, Dijana Miceva

Faculty of Medical Sciences, University “Goce Delcev” – Stip, Krste Misirkov 10-A, 2000 Stip, N. Macedonia

Introduction

Typically for the entire pharmaceutical system is complex legislation. The structure of drug regulation needs to be continually evolved to respond appropriately to the growing challenges of society.

The population served by one pharmacy varies from 18,000 in Denmark to 1,000 in Greece. In the Republic of North Macedonia in 2012 one pharmacy serves around 3,000 inhabitants, which has been established as the maximum number of pharmacies in accordance with the Regulation of Health Institution Network. According to current published population and number of pharmacies, in 2019 one pharmacy serves about 1800 inhabitants.

The purpose of this paper is to review and analyze the regulation related to public pharmacies voted in the last decade and to see the current legal status, with a discussion of specific solutions and suggestions for possible improvement.

Materials and methods

The laws, bylaws, regulation and decisions from 1992 to 2020 were used to regulate the operation of public pharmacies.

The materials were reviewed and have been chronologically analyzed and sorted by impact on the general status of pharmacies. A comparative method and a compilation method were used.

Results and discussion

Retailing of medicines in the Republic of North Macedonia is carried out at primary health care institutions - pharmacies and hospital pharmacies within the hospital health institutions. The authorization for doing business is regulated by the Law on Medicines and Medical Supplies (as Retail Trade) and the Law on Health Care (as a health care institution), and in accordance with the bylaws of the Law on Health Care. The Law on Medicines and Medical Devices from 2007 to 2019 has 17 amendments and additions. The pharmacy is a health institution and the establishment and activity of the pharmacy is carried out in accordance with the Law on Medicines and Medical Devices and the Law on Health Care, and the approval for performing the activity of the pharmacy is issued by the Minister of Health for an indefinite period. It has public service status, which is not always respected when issuing approvals.

Amendment to 2013: categorization of pharmacies in terms of space, equipment and staff - refers to settlements in rural areas and goes into the competence of the Ministry of Health (MOH).

Amendment to 2014: Pharmacy founder can only be a pharmacist (abolished by Constitutional Court Decision); allowed retailing of drugs outside the pharmacy; owners who own more than two pharmacies are required to open a pharmacy in a rural area.

Amendment to 2015: changed the name of the law to the Law on Medicines and Medical Supplies; stricter penalties for the sale of a drug classified as a prescription drug; a distance of at least 100 meters

* bistra.angelovska@ugd.edu.mk
from one pharmacy to another for low density populated areas; permitted retail supply of prescription drugs at the store or at a gas station; The Director of the Agency shall prescribe the procedure, conditions and manner of obtaining a retail marketing authorization by a by-law which has not yet been voted.

From 2010 to 2018, three changes were made to the implementation of act to have a pharmacist in each shift.

From the Health Care Law in 1991 have been defines the pharmaceutical activity as part of the health activity, the pharmacist as a health worker and the pharmacy as a health institution. The competent authority for approval of activity is the Ministry of health. The authorization is issued in accordance with the Health Care Law and the Law on Medicines and Medical Devices, and the closer conditions regarding space, equipment and staff are regulated by a Rulebook voted in 1992 and amended in 2005.

Amendment to 2004: privatization of the pharmacy activity. The profile of the founder / owner of the pharmacy is not specified, but the carrier must be a pharmacist.

In 2012, a new one law was voted, and that one is undergoing 18 changes and additions by 2019. The pharmacy is regulated identically as in the old law.

In the new Rulebook on space equipment and staff voted in 2013 the requirements are almost identical to those prescribed in the Rulebook from 1992.

The list of health services that can be provided at a pharmacy is based on the definition of a pharmacy from 1977 year. It was adopted at the end of 2013 and has not been updated nor adapted to the contemporary needs of the health system and patients

With the Methodology on the Formation of Unique Drug Prices (2011), a by-law of the Law on Medicines and Medical Devices, the price of the service is marginal, scalable and regressive depending on the reference price, on average around 15.00 denars per issued drug. Amendment to 28.10.2019 - the service is calculated as 18% margin on the invoiced medicines at a reference price, but not more than 180.00 denars, on average around 17.00 denars by prescription issued.

The cost of the service should not be related to the cost of the drug, but to the service provided. It is necessary to revise the List of services provided at the pharmacy and standardize it according to the resources consumed (material, human by qualification and time) to obtain a realistic cost of service, to improve the quality and the final outcome of therapy.

**Conclusion**

Frequent and inconsistent amendments to laws and bylaws, often with inadequate changes and interference with the powers to implement them, untimely voting of bylaws and poor coordination between laws regulating the sector create confusion and make it difficult to implement. None of the changes made to the implementing laws and bylaws penetrates the core of the business and does not improve the status of pharmacies. This prevents the development of modern practices such as those in developed countries that have proven to save financial resources on funds, citizens and society, while improving the outcome of therapy and public health. It is necessary to vote a Law on Pharmacy, to redefine the activity and list of services, to vote a unique methodology for pricing and to change the way of payment of the pharmaceutical service according to the service rendered and the resources.

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Safe and effective medicines for all - one day project in Serbian pharmacies

Tatjana Šipetić¹, Ivana Tadić²*, Dragana Rajković¹, Sandra Vezmar Kovačević², Milica Ćulafić², Tatjana Milošević³, Jelena Stefanović Vojinović⁴, Suzana Marinković⁵, Mika Simišić⁶

¹The Pharmaceutical Chamber of Serbia, Mutapova 25, 11000 Belgrade, Serbia
²University of Belgrade – Faculty of Pharmacy, Vojvode Stepe 450, 11000 Belgrade, Serbia
³Community Pharmacy Melem, Zanatlijska 32, 15000, Šabac, Serbia
⁴Community Pharmacy Lilly, Pilota Mihajla Petrovica 6, 11000 Belgrade, Serbia
⁵Community Pharmacy Zaječar, Nikole Pasica 11-13, 19000 Zaječar, Serbia
⁶Community Pharmacy Subotica, Matije Gupca 26, 24000 Subotica, Serbia

Introduction

The traditional roles of pharmacists are dispensing of Rx medicines and other medicines groups, care of patients, monitoring of medicines utilization, preparations of medicines (small-scale manufacture of Rx medicines), provision of traditional and alternative medications, care of patients’ minor ailments, disease prevention and health promotion and informing other health care professionals (WHO Consultative Group, 1988.). In the previous period, these roles experienced significant changes. Pharmacists became professionals who not only provide/dispense medications to patients but cooperate more with other health care professionals about patients’ therapy. Cooperation with other professionals overcomes the basic role of pharmacists (informing of other health care professionals) and leads up to collaborative practice with the patient as the center of care (Kehrer et al., 2013). The new role of pharmacists in medication optimization contributes to reduction in health care costs and savings of the healthcare public funds (Dalton and Byrne, 2017).

The International Pharmaceutical Federation (FIP) provides support to pharmacists globally. With the aim to enhance the position of the pharmacists within the society and inspire pharmacists to be more involved in improving health of communities, the FIP encourages pharmacists to organize activities during the World Pharmacist day. The theme of the FIP World Pharmacists Day in 2019 was “Safe and effective medicines for all”. In line with the defined theme, Pharmaceutical Chamber of Serbia organized the one-day project in community pharmacies nationwide. The aim of this paper is to present all services that pharmacists provided during this project.

Materials and methods

All members of the Pharmaceutical Chamber were invited to voluntarily participate in the project. During the World Pharmacists day (25th of September 2019.) pharmacists provided different
services to patients’ and all of these services were documented using the specially designed form. The form was designed by the Pharmaceutical Chamber expert group and first was tested on small sample of pharmacists.

Results and discussion

The results include data form 93 pharmacists who completely filled the form. They provided services to 536 patients. The average number of patients per pharmacist was 5.76 and 6.62 per community pharmacy. Patients who received the pharmacists’ services were 64.99±12.81 years old and mostly female (57.28%). The average number of medicines per patient was 4.55±2.19 and mostly belonged to C category using the ATC classification.

During the project pharmacists documented all medications, dietary supplements (DS) and other pharmacy products that patients used or were being prescribed to patients. The most of medications that patients used were being prescribed to patients by medical doctors (MD) (91.37%, mostly Rx medicines), recommended by pharmacists (1.44%, mostly OTC medicines) or used for self-medication (1.64%, mostly OTC medicines). Dietary supplements were used by 59.33% patients and in average 1.66 DS per patient. DS were predominantly recommended by pharmacists (52.80%), or were used by the opinion of patients themselves (28.24%), or recommended by MD (18.95%).

The most of pharmacists checked if the medicines regimen was appropriate (94.78%) and if the medicines usage is correct (89.07%). During the medication review of patients’ therapy pharmacists documented clinically important drug-drug interactions in 18.19% patients. They also notified adverse drug reactions in 2.54% of patients.

Other pharmacists-led interventions that were provided to patients were: Nonpharmacological interventions (education about lifestyle, such as advice to patients on healthy lifestyle, including diet, weight management, alcohol consumption and smoking cessation), counseling on regular measurements of clinical parameters, demonstration of correct use of medical devices, provision of advices about correct medicines storage and proper medicines’ application (82.09%, 72.20%, 17.91%, 48.13% and 37.13% prospectively).

Conclusion

The study results indicated that pharmacists are highly motivated to provide specific interventions to patients. During the World Pharmacists day pharmacists have devoted more time to each patient and invested effort to help patients. These pharmacists-led interventions contributed to encouragement of pharmacists to improve health of patients, as well as to strengthening of the pharmacy profession.

References

Approaches for regulation of the “off-label use” within the European Union

Maria Drenska*, Ilko Getov

Faculty of pharmacy, Medical University Sofia, 2 Dunav str., 1303 Sofia, Bulgaria

Introduction

The European Medicines Agency defines the off-label use as “situations where a medicinal product is intentionally used for a medical purpose not in accordance with the authorised product information”. In other words, off-label use can be defined as the use of an authorized medicine in an unauthorized manner, which raises many questions about the risk, ethics and legality of this type of practice.

Studies show that about 40% of medicines in adults and up to 90% in children are used off-label (Gazarian et al., 2006). Some groups of patients are most affected, such as children, mental patients, cancer patients and patients with rare diseases where there is simply no alternative treatment with available authorized medicinal products (Weda et al., 2017).

Despite increasingly stringent regulations, off-label use remains a weak point in today's pharmaceutical legislation. In recent years, guidelines and legal changes in some countries have defined conditions and responsibilities, but in most European countries there are still no rules for off-label use (Drenska and Getov, 2017).

The purpose of this study is to identify and analyze different off-label regulatory approaches adopted by some Member States and to summarize efforts to establish a common harmonized approach to regulate this practice within the European Union (EU).

Materials and methods

The subject of our research was Bulgaria and selected European countries (France, Italy, Spain, Germany and the United Kingdom), which have specific solutions to regulate this practice. Studied and analyzed were legal documents, manuals, expert opinions, scientific publications and other available information in PubMed, Google Scholar and Google, without limits in the time range.

Results and discussion

From all the countries studied, only in Bulgaria there are no clear rules for the off-label use of medicines. The measures taken by the other selected EU countries, are showing significant differences and a specific approach. Some are within the scope of pharmaceutical legislation, others within the national health insurance legislation, and some are guidelines of national medical associations.

The approaches taken by the United Kingdom and Spain give to doctors some freedom to use medicines off-label, but in certain conditions (lack of alternative treatment and / or informed consent from the patient).

In Italy, France and Germany, another approach has been taken. The off-label use is only allowed if
they have gone through a specific approval procedure and the lists of medicines that can be used off-label are maintained by the relevant national medicine agencies.

Explicit informed consent from the patient is required in two of the countries - Spain and Italy, and in the United Kingdom, according to the prescribing guidelines, is advisable.

The analysis shows that, different approaches taken by these EU Member States have their advantages and disadvantages.

However, we found the approach in Spain to be the most comprehensive and effective, with the following arguments:

- Gives freedom to doctors in their work;
- Makes it possible to treat patients in urgent need;
- The fundamental right of the patient to be informed and to participate in treatment is guaranteed;
- Allows doctors to avoid liability associated with the off-label use;
- Protects authorized and clinically proven medicinal products;
- Ensures proper use and reduces risk.

**Conclusion**

The regulation of off-label use is highly recommended. The lack of rules for off-label use leads to uncertainty among doctors and to patient dissatisfation. The presence of rules, facilitates patient access to medicines, increases the number of medicines doctors have and addresses many of the problematic aspects associated to this practice (e.g. safety, ethics, legality, etc.).

Finding a common solution for regulation of the off-label use across the EU, would harmonize the different approaches in different countries, but also would help others to impose control on this problematic practice.

**References**


The impact of clinical pharmacy-led medicines management support for patients with COPD

Olivera Krstic Nakovska¹*, Dejan Dokic¹, Dimitar Karkinski¹, Sava Pejkovska¹, Elena Janeva¹, Sanja Filkova², Zorica Naumovska³, Aleksandra Kapedanovska Nestorovska³, Ljubica Suturkova³

¹University Clinic of Pulmonology and Allergology, Majka Tereza 17, 1000 Skopje, North Macedonia
²Public institution for the needs of University Clinics, Institutes and Urgent Centre, Department of Clinical Pharmacy, Majka Tereza 17, 1000 Skopje, North Macedonia
³Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000, Skopje, North Macedonia

Introduction

Chronic obstructive pulmonary disease (COPD) has been defined as a non-fully reversible airflow obstruction, characterised by breathlessness. It is mainly caused by smoking or exposure to pollutants. The goals of COPD treatment are to slow down disease progression, limit symptoms, increase overall health and prevent and treat flare-ups (GOLD, 2020). Inhaled therapies are the mainstay of treatment. Bronchodilatators (short and long acting) have an established role in the management of COPD and significantly reduce the risk of adverse outcomes such as COPD exacerbation. The addition of inhaled corticosteroids may benefit patient who remain symptomatic despite regular treatment with long acting bronchodilatators. The patients should be trained for utilization technique before they are prescribed inhaler by the doctor. Very few published studies focus on adherence in therapy to treatment regimes in COPD, but evidence suggests adherence is poor (Alton and Farndon, 2018). Clinical pharmacist can play an important role in identifying and instruct COPD patients in their inhale techniques to help to improve symptoms burden and medication compliance and overall outcomes (Khdour et al., 2009). The aim of this study is to assess the impact of clinical pharmacy-led management support in patients with COPD in The Clinic of Pulmonology and Allergology of Skopje, Republic of North Macedonia.

Materials and methods

Observational, longitudinal and prospective study was conducted in The Clinic of Pulmonology and Allergology of Skopje, Republic of North Macedonia. Patients were verbally informed before they were included in the study and 32 eligible hospitalized patients (29 men and 2 woman), over 40 years old, with a history of COPD were recruited. Twenty-two patients were assigned in the intervention group and 10 patients were assigned in the usual care or control group. The patients were followed from March 2019 to December 2019 and each of the patients completed COPD assessment test (CAT) and customized Morisky medication adherence scale at baseline, after 3 months and after
6 months. COPD assessment test (CAT) is designed to measure the impact of COPD on a person’s life and how this changes over time. It consist of eight simple questions that most patients should be able to understand and answer easily. CAT scores are in to 0-40 range, 0 being very low impact symptoms severity. The customized Morisky adherence scale consists 8 questions that reflect the number of ways medication omission can occur: forgetting, carelessness, stopping when feeling better.

Adherence scores are 0-40 range with 0 being high adherence and 40 low adherence. Intervention patients were educated on disease state, medication and breathing techniques and they were followed up at 3 and 6 months during a scheduled phone visit.

The intervention of clinical pharmacist on every visit was medication review and follow up as appropriate, reassess inhaler technique, further advice and support and fulfill the questionnaires. The patients from the control group received usual hospital inpatient care, from medical and nursing staff, but did not receive the structured intervention by the clinical pharmacist referred above and they fulfilled the questionnaires in 3 and 6 mounts period.

Results and discussion

The total number of patients that finished the study was 21 in the intervention group and 10 from the control group, because one patient from the intervention group has died during the study. As the study was conducted in the hospitalized patients an overall improvement of the CAT questionnaire and the customized Morisky adherence scale was confirmed with a significant improvement in the intervention group. In the intervention group the COPD assessment test CAT decreased with a significant difference from 56% (22.4) at baseline to 22.3% (8.9) after six months and from 63.5% (25.4) at baseline to 47.75% (19.1) at six months in the control group. There was a significant difference between the intervention group and the control group regarding the adherence scale. The confirmed difference was 30.1% (12.04) vs 21.25% (8.5) at baseline and 8.75% (3.5) vs 20.25% (8.1), after six months in the interventional group in comparison with the control group. The study has confirmed that implementation of the action plan consisting the disease management skills explanation, inhaler technique education led by the clinical pharmacist, was effective and resulted in improved patient satisfaction with improved symptoms management and improved overall outcomes for the intervention group compared with the control group. This was a short time limited study focused on education regarding the disease, inhaler technique and assessment. Adherence results should be treated with caution due to the small patient number and further evaluation is needed in order to assess the impact of this intervention on exacerbation, hospital readmission.

Conclusion

The clinical pharmacy led intervention can contribute to improve adherence to medication and improve better disease control in COPD patients. Patients have appreciated the time taken by the clinical pharmacist and found face to face contact invaluable, results in improvements in inhaler technique.

References


Satisfied customers and Good Commercial Practice challenge for the pharmacy law in the cosmetic industry

Katerina Ancevska Netkovska1*, Jadranka Dabovikj-Anastasovska2

1Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Tereza 47, 1000 Skopje, North Macedonia
2Iustinianus Primus Law Faculty, Ss. Cyril and Methodius University, bul. Goce Delchev 9b, 1000 Skopje, North Macedonia

Background

The Pharmaceutical Law, specifically the part of this law that provides the legal framework for cosmetic products in the EU, comprises of rules for the production, safety, distribution and marketing of cosmetic products. We assess that the legal regulation of the cosmetics industry is particularly important because: first, there is a significant expansion of the industry; second, these products are separated by a thin line from the group of products considered to be medicinal products; and third, after the food industry the cosmetics industry is the one that has the largest number of consumers in the world.

Research shows that the cosmetics industry is expanding in the EU. In our country, there are number of small and medium-sized companies in the field of production, especially of organic cosmetics. Of course, the largest producer and exporter remains Alkaloid AD Skopje.

It is considered that the vast majority of Europe's 500 million consumers use cosmetics and personal care products (hereafter 'cosmetics'). European consumers spend, on average, € 135 per year purchasing different cosmetic products.

Ensuring equal rights for consumers in the common market and protecting consumers from unfair business practices at all stages of commercial relations between traders and consumers i.e. prior to the sale, during the transaction and after the sale is particularly important when it comes to consumer rights in the European Union.

In this paper we analyze the EU legal framework for cosmetic products, with purpose to answer whether this legislation provides adequate and satisfactory consumers’ protection in the common market. The analysis also aims to show that specific sectoral rules, combined with horizontal consumer protection rules, can to some extent provide for the prevention of unfair business practices. Besides having a comprehensive legislation, it is certainly very important to implement this legislation in practice so as to reach the required levels of consumer protection and competitiveness. In this regard, it is to be noted that other branches of law such as Intellectual Property Law and Competition Law contribute to the achievement of the set goals.

Therefore, the comparative legal method was used to assess the level of alignment of the national legislation with the relevant EU regulation of cosmetic products.

Results and discussion

The aggressive and deceptive practices have been present mostly in the electronic trading, but research has shown a violation of the rules as to the accuracy of the claims and the rules on advertising
in all types of sales (COM (2016) 580 final). One of the problems that are considered particularly acute in the EU is when cosmetic products are attributed with medicinal effects. Most Member States identified claims that a cosmetic product has medicinal functions or curative or biocidal effects, although the responsible person could not give evidence to support this, have the most dangerously misleading claims for consumers. Believing that a cosmetic product has therapeutic effects and medicinal properties could lead consumers to delay adequate medical treatment (Regulation (EU) No 655/2013, Guidance Document).

The Regulation (EC) No 1223/2009 on cosmetic products and Directive 2005/29/EC on business-to-consumer unfair commercial practices (UCPD) have a similar objective, to protect consumers from misleading claims, and the latter may apply in a complementary manner to cosmetic claims, to the extent these qualify as a commercial practice within the meaning of the UCPD. The provisions of the Cosmetics Regulation, as lex specialis, prevail over the UCPD where the specific aspects of unfair commercial practices are regulated by the former. That principle is clearly established in the UCPD, which provides in its Article 3.4 that in the case of conflict with 'other Community rules regulating specific aspects of unfair commercial practices' the latter shall prevail and apply to those specific aspects. That principle is further clarified in recital 10 of the UCPD which states that the 'Directive accordingly applies only in so far as there are no specific Community law provisions regulating specific aspects of unfair commercial practices, such as information requirements and rules on the way the information is presented to the consumer.

In the Republic of N. Macedonia, in the process of adoption of the new Law on Consumer Protection as a general law, and more specifically in the case of the products and services, for which there are specific departmental rules, there was a question of whether this law shall provide for the implementation of the supervision and enforcement of the rules of the general law by the competent departmental bodies, especially the unfair commercial practice, or whether they can be performed by the state market inspectorate. In the final version of the draft LCP, the standpoint that the supervision and implementation of unfair commercial practice will be performed by competent departmental bodies prevailed. In our case, regarding cosmetic products, the competent departmental body is the State Sanitary and Health Inspectorate. The afore-stated shall be valid only if the practice is unfair in relation to claims for cosmetic products. With respect to the prices or certain forms of advertising and aggressive commercial practices the responsible authority shall be the State Market Inspectorate. This separation of the supervision and enforcement responsibilities sometimes gives negative effects because, either both bodies consider themselves to be competent, or both regard themselves as not competent.

**Conclusion**

Further efforts should be made on national level for the approximation of the national legislation and practices. The competent authorities should have more proactive role in protection of the consumers of cosmetic products. The level of knowledge and skills of the relevant business sector should be increased so as to provide adequate services to the consumers but also to take measures against competitors who act unfairly and distort the competition.

**References**


Changes in intellectual property systems in Commonwealth of Independent States and their harmonization within Eurasian Economic Union

Marija Mitkovska¹*, Katerina Anchevska Netkovska², Ana Poceva Panovska²

¹Alkaloid AD Skopje, Blvd. Aleksandar Makedonski 12, 1000 Skopje, North Macedonia
²Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Teresa 47, 1000 Skopje, North Macedonia

Introduction

Intellectual property aspects, predominantly patent rights and data exclusivity, directly influence access of medicines. Considering this, EU and USA have well balanced intellectual property systems (IPSs). In comparison with Commonwealth of Independent States (CIS) and Eurasian Economic Union (EAEU), the situation is opposite. CIS have constantly fluctuating IPSs, where some of the countries are implementing EU directives, and others are moving towards US IPS. On the other hand, EAEU is moving towards unifying the laws of intellectual property and medicines, thus arising the needs of harmonization the national laws of the member states.

In this article, we put focus on data exclusivity and patent linkage systems in the member states of EAEU and within EAEU.

Materials and methods

Method 1 Patent linkage and data exclusivity review

Analysis and comparison of regional regulation of EAEU and national legislations of the member states using the EAEU Rule and national patent laws and laws of medicine.

Results and discussion

Data exclusivity, as a power intellectual property right, gives originators data protection of pre-clinical and clinical trials data for a certain period, which differs among countries. During this period, national authorities are forbidden to accept generic applications. In some jurisdictions, additionally to data exclusivity, is given a market exclusivity period. During the period of market exclusivity, submission of generic applications is permitted, only the launch of the generic products is forbidden (McKenzie, 2019; Хабриева, 2019). Generally, member states of World Trade Organization (WTO) are obliged to implement this provision on a national level (Хабриева, 2019).

Patent linkage is a practice of linking the marketing approval procedure for a generic medicinal product to the status of a patent of the originator reference product. Patent linkage system differs among countries. The health authorities, depending on the jurisdiction in question, may refuse the application, refuse to grant a marketing authorization, inform the patent owner to enable it to take any relevant action, etc. As a part of the registration dossier, among other administrative documents, a non-infringement statement has to be submitted (McKenzie, 2019).
Armenia has implemented data exclusivity regime, giving originators 8 years of data exclusivity and 2 years of market exclusivity from the first marketing authorization of the originator product in Armenia or in a member state of the international professional organization established by the Government (The Republic of Armenia Law, 2016). Following the EU regime, the patent linkage provision was abandoned last year in June.

Belarus is a country which is still not a member state of WTO. Therefore, data exclusivity regime is not implemented in the legislation (ICO East Europe and Central Asia Union of PLWH, 2013; Хабриева, 2019). On the other hand, patent linkage provision is implemented in a non-typical way. A non-infringement statement letter is a part of the registration dossier, but the health authorities are not obliged to control the validity of those statements. In case of noticing that the registration dossier contains false information, the health authorities may decide to suspend the registration procedure/issued certificate for not more than 6 months (ICO East Europe and Central Asia Union of PLWH, 2013).

Kazakhstan has adopted data exclusivity in November 2015, so the national legislation could be aligned with WTO agreements. The data exclusivity period for an originator product is 6 years from the date of the first marketing authorization in the country (Abylkhanova, 2018; Хабриева, 2019). Before adopting data exclusivity, there was a good practice of the Kazakh patent linkage system. After November 2015, there is no longer a clear-cut situation regarding the implementation of the patent linkage system (McKenzie, 2019).

Kyrgyzstan has abolished the patent term extension, so the patents will be valid maximum for a 20 years period from the submission of the patent application (Lidings, 2020). Currently, data exclusivity and patent linkage system are not implemented in the national legislation (MeTA Secretariat Kyrgyz Republic, 2012).

Russia has implemented data exclusivity in 2014 (final provision introduced on 1st of January 2016), giving originators a 4 years of data exclusivity and 2 years of market exclusivity from the first marketing authorization of the originator product in Russia (Хабриева, 2019). In January 2020 is announced that, a patent linkage system, as the USA system, is going to be implemented (Lidings, 2020).

EAEU has adopted data exclusivity provision. The data exclusivity period is 5 years from the first marketing authorization of the originator product within the union and 1 year of market exclusivity. Patent linkage system is also applicable within EAEU. In the registration dossier would have to be included information on the patents protecting the generic drug and a non-infringement statement (Совета ЕЭК, 2016). Additionally, within EAEU, a unified patent system is going to be introduced (Lidings, 2020).

Starting from 2021, national marketing authorizations of the EAEU member states will be abandoned. There will be the possibility only for regional marketing authorization of the drugs within EAEU (Совета ЕЭК, 2016).

Conclusion

There is inconsistency between the national legislations of the EAEU member states and the regional regulation of EAEU in terms of data exclusivity and patent linkage. It is obvious that EAEU regulation moves towards US patent system. Since starting from 2021 only regional marketing authorizations will be possible within EAEU, it is necessary to make amendments of the national laws of EAEU member states to be align with the supranational regulation of EAEU.

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Attitudes and practice of pharmacists in pharmaceutical waste management – a pilot study in the city of Novi Sad

Svetlana Stojkov*, Nataša Jovanović Lješković, Milan Ilić, Jovana Vasiljković, Slobodan Gigov

* University Business Academy in Novi Sad, Faculty of Pharmacy, Trg mladenaca 5, 21000 Novi Sad, Serbia

Introduction

Development of pharmaceutical industry and constant improvement of healthcare systems have significantly increased availability of drugs worldwide. These phenomena, together with presence of consumerism in all segments of society, undoubtedly lead to increased procurement and use of medicines. Furthermore, this cause continuous increase in production of drugs globally (IMS institute, 2017), health complications as a result of inadequate drug use, and presence of pharmacicals in our environment with numerous ecological and health consequences (WHO, 2011). Pharmacists, as experts for medicines, have significant responsibility for health of the whole society, being involved in scientific research and development of new drugs, production of medicines and their distribution, patient counselling on proper drug, safe storage of drugs, and finally, appropriate pharmaceutical waste disposal.

Preservation of environment requires interdisciplinary and intersectoral collaboration on global and local level.

The aim of this paper is to show results of the collaboration project of the Faculty of Pharmacy, the Faculty of Law for Commerce and Judiciary, both members of University Business Academy in Novi Sad and local government of Novi Sad and comprehensive analysis of the pharmaceutical waste management process in the territory of Municipality of Novi Sad.

Materials and methods

Sectional study was conducted in pharmacy chains and individual community pharmacies in Municipality of Novi Sad by specially designed questionnaire. The questionnaire consisted of 12 questions, out of which two were open-ended questions and ten were closed-ended questions. Participation in this research was voluntary and anonymous.

Results and discussion

87 community pharmacists from Municipality of Novi Sad participated in this research. Average length of pharmacists’ working experience is 13 years. Amongst total number of respondents in this research, 16.1% had postgraduate degree and 29.9% of them were at the position of pharmacy manager. Most of pharmacists (89.6%) provide information about safe and proper disposal of unused medicines in households to their patients, 37.9% of pharmacists
provide such information in every appropriate situation, and 51.7% of them do this at the request of the patient. About 10% of respondents do not provide these information to patients. Most of respondents stated that the information about safe disposal of unused drugs from households are required several times in a month (36.8%). 34.5% of pharmacists said that they provide such information several times in a year, 16.1% of them do it every day, while 12.6% of pharmacists stated that their patients do not ask for information about unused drugs disposal. According to previous study in this region, knowledge and habits of citizens of Novi Sad regarding pharmaceutical waste disposal are not satisfactory – over 85% of respondents dispose it as communal waste (Kusturica et al., 2012), which is higher rate than in recent study from Poland, where 68% of drugs end up in communal waste and sewage (Rogowska et al., 2019).

Almost all of respondents (98.9%) consider that pharmacists are competent for providing information about appropriate pharmaceutical waste disposal, and 96.6% of them stated that unused drugs and expired medicines belong to hazardous waste. Regarding question whether community pharmacies have to receive unused and expiry medicines from citizens, 13.8% of pharmacists gave negative answer, 3.4% of them were not sure, and 5.8% of them do not know the answer. Different level of knowledge of pharmacists about this topic result in various practice in pharmaceutical waste management in community pharmacies in Serbia (Stojkov et al., 2018). Most of pharmacists, 95.4% of them, consider that there is a need for education about safe pharmaceutical waste disposal. According to the results of this study, 71.3% of respondents would attend such education, 23.0% of them responded “perhaps”, while 5.7% said they would not attend education on this topic.

Conclusion

In order to reach high standards in pharmaceutical waste disposal collaborative environment including citizens, healthcare professionals and media should be improved. Activities in this process should start with education of citizens and continuing professional development of pharmacists, which would result in appropriate pharmaceutical waste management among citizens and pharmacists.

References


Intellectual property rights and drug advertising in Republic of North Macedonia

Biljana Nestorovska Gjoshevska, Katerina Ancevska Netkovska*, Marija Glavas Dodov

Faculty of Pharmacy, Ss. Cyril & Methodius University, Majka Teresa 47, 1000 Skopje, North Macedonia

Introduction

In a time of rapid development of technology and eased communication, patients’ needs more information for the medicinal products they used or could be used, however, pharmaceutical companies are limited by regulative with information’s and the ways they can provide/present their products.

Under Directive 2001/83/EC, “advertising” includes a wide range of activities that are designed to promote the prescription, supply, sale or consumption of medicinal products. There is a general prohibition on advertising prescription-only medicines (“POM”) to the general public, although it is permissible to advertise non-POM products, such as over-the-counter pain relief medication (ICGL, Pharmaceutical advertising, 2018).

The variety of innovative digital advertising techniques in the online environment has created new opportunities for companies to expand advertising beyond its traditional supporting role for a good or service. At the same time, the Internet and digital technologies have created new potential problems because of the ease and speed with which advertising content can be copied, assembled, reshaped and distributed worldwide.

As in any creative and/or innovative industry, advertising companies are also faced with copycats, illegal use of their creative ads, products and contents by unfair competitors. It seems logical that under such circumstances, companies will act to protect their creative achievements against unfair or illegal use by others. In this context, the intellectual property (IP) system offers various possibilities which advertising companies can and should use.

There is a number of IP issues related to creativity and advertising, such as how advertisers can protect their unique and original creations as intellectual property rights (IPRs); how advertisers can use registered trademarks; or the dangers of violating the IP rights of others while creating or using advertising content in a traditional or digital environment (WIPO, Managing IP and Advertising, 2011).

The aim of this study was to evaluate the IP rights and drug advertising in the R.N. Macedonia (RNM) by on-line prepared questionnaire for different population and professional structure groups.

Materials and methods

For the purpose of researching IP issues related to pharmaceutical advertising in RNM we started an exploratory research on this topic worldwide. We used method of “Collecting secondary data”, first. Using Internet search engines, a comprehensive literature review was carried out to find relevant studies performed on this topic. No other study was found to be conducted in RNM. Similar and valuable for this research were studies published in 2013 and in 2016 by EUIPO committee: European Citizens and IP perception awareness, and behavior. Also, several studies for deceptive advertising was found and used as data to continue with our further research, “Descriptive research (primary) data”.

* kaan@ff.ukim.edu.mk
Descriptive research was performed after collecting sufficient secondary data. We expected by gathering information and estimation about the perception, awareness, behavior and knowledge of the citizens of RNM for IPR and drug advertising issues will help us to measure the influence on IP on advertising of drug products from one side and analysis of regulative on the other side.

First, pilot survey was prepared in order to predict possible problems during the online interview.

The interview questionnaire (Google Docs. Forms) included 22 items related to measurement of the perception on IP, different drug advertising aspects and prevalence of misleading advertising. The snowball effect of questionnaire was planned (Maysonnave and Delorme, 2013).

The survey was conducted during May and June 2019 on 314 residents of RNM Participants of different age groups, social categories, professional structure, education level and nationality.

Obtained data were tabulated using Microsoft Excel® (Microsoft Corp. Redmond, WA, USA), computed and consequently evaluated using statistical software STATGRAPHICS Centurion XVI evaluation (Stat Point technologies Inc., USA).

Results and discussion

Participants of different age groups, social categories, professional structure, education level and nationality were interviewed.

The findings indicated that 63% of the surveyed participants have more trust in famous branded products than in smaller on non-branded products. The 68% of the participants claim that the trade mark has influence on their choice which drug to consume. 60% of the respondents think that they can recognize which advertisement is misleading.

It is interesting to notice that 46% of surveyed participants do not believe in truthfulness of advertisements in RNM, 46% think that non-fair concurrent advertising exists in RNM and additional 29% have opinion that most frequently there is non-fair concurrent advertising in RNM.

58% of the respondents answered correctly that only OTC pharmaceutical products are allowed to be advertised in RNM and 53% think that in RNM advertisement are controlled by authorities before launched.

When asked in which pharmaceutical advertiser believes the most, 30% of the respondents claimed that they believe in truthfulness of RNM pharmaceutical advertisers and ~ 63% believe on European advertisers mostly.

Conclusion

The findings indicate that Intellectual property rights have influence on the drug advertising in RNM.

This study might be base for following RNM citizens’ reaction on intellectual property, drug advertising and misleading advertising as a present world global problem. Further researches might be valuable for need of alignment of the regulative on international level.

References


Safety limitations of fluoroquinolones’ use

Violeta Getova1*, Ilko Getov2

1Bulgarian Drug Agency, 8 Damyan Gruev str., 1303 Sofia, Bulgaria
2Faculty of Pharmacy, Medical University Sofia, 2 Dunav str., 1303 Sofia, Bulgaria

Introduction

Fluoroquinolones are class synthetic antibacterial medicines in use since 1961. First representatives had a rather narrow antibacterial spectrum. With the inclusion of the fluorine atom in the molecule the spectrum of antibacterial activity had significantly broaden. Nowadays fluoroquinolones are separated in four generations: first and second generations are mostly active against Gr(-) microorganisms and third and fourth generation are effective against Gr(+) as well. Fluoroquinolones play a significant part in the treatment of respiratory, gastrointestinal and urological infections and are mostly used per os.

This makes them accessible and preferable therapeutic option but also brings up questions about control of use, overuse and abuse. Aside from the growing problem of microbiological resistance, a lot of severe adverse drug reactions have been linked to fluoroquinolones treatment recently.

Materials and methods

For the purpose of the current study, a review of the referral procedure of the safety of quinolones and fluoroquinolones has been done. The official assessment report of the Pharmacovigilance Risk Assessment Committee (PRAC) of the European Medicines Agency (EMA) and the documents of the procedure were analyzed. The data shown by the PRAC was amended with a literature search on the topic of safety limitations of fluoroquinolones use. The main safety concerns were outlined and both the non-clinical and clinical aspects of their development were studied. Along with the regulatory point of view of the PRAC assessment we also used a practical approach to point out the biggest issues related to fluoroquinolones therapy as well as a brief guidance on therapeutic behavior in cases where quinolone therapy is no longer the best option based on the latest safety information.

Results and discussion

The procedure of safety assessment and PRAC referral of quinolones and fluoroquinolones was conducted in 2018. For the purpose of the referral the EudraVigilance database was searched. All ADRs reported to the system in the period 1998-2016 which included quinolones or fluoroquinolones as suspected drug were extracted. Other criteria of the search were serious ADR which led to long term complications, ADR with duration more than 30 days or ADR with result “recovered with sequels”. The total number of cases that met the criteria was 2141. 393 of them were marked as leading to disability. The analysis showed that the most frequently reported medicines were levofloxacin, ciprofloxacin, moxifloxacin, ofloxacin and norfloxacin. The majority of the cases referred to impairment of musculoskeletal system, nervous system and general disorders with 37% of the cases including ADRs in more than one system. Most numerous ADR cases were tendon inflammation, tendon rupture, arthralgia and tendon disorder.

Non-clinical, clinical studies and scientific publications present enough data to consider that...
there is drug-reaction relatedness between use of fluoroquinolones and potentially disabling ADR of the musculoskeletal system (Lewis & Cook, 2014). 46% of the analyzed cases represented problems with muscles and mainly tendons. 15% of the analyzed cases were ADRs from the nervous system and most frequently reported ADRs were sense and memory impairments. Central nervous system damage such as sleep disorders, depression and anxiety were also frequently reported ones, and 5% of them were marked as resulting in disability (Doussau de Bazignan et al., 2006; Tome & Filipe, 2011).

Over 100 indications for use of fluoroquinolones were present in the EU at the start of the referral. During the safety assessment they were classified in four categories based on data on the risk/benefit ratio. In the first category were put those indications where no change in treatment behavior is needed. This group includes chronic sinusitis, cervicitis, orchitis, gastrointestinal infections, intraabdominal infections etc. In these indications PRAC considers that the benefit outweighs the potential risk of disabling ADRs (EMA, 2018).

The second group of indications includes acute sinusitis, uncomplicated cystitis, otitis media and COPD exacerbations. For those conditions PRAC considers there are other medicines present in the EU that have a greater benefit for the patient and should serve as first line treatment choice. For this reason, uncontrolled use of fluoroquinolones in this subset of indications carry risk of development of microbial resistance.

For the third group indications PRAC’s recommendation is to suspend use of fluoroquinolones because of a negative benefit/risk ratio. In pharyngitis, tonsillitis, laryngitis, vaginal infections, septicemia and endocarditis etc. fluoroquinolones are not recommended as treatment option. The majority of the cases of pharyngitis, tonsillitis and laryngitis are of a viral nature rather than a bacterial pathogen and this makes treatment with quinolones unnecessary (Reveiz & Cardona, 2015). In cases of septicemia PRAC recommends start treatment of the underlying cause for septicemia which usually requires antibiotic treatment.

In the fourth group are put some indications which are considered too vast and unspecified. Rewording should be done and the product information should be corrected in order to include the updated guidance for use of fluoroquinolones.

As a result of the PRAC safety referral procedure the benefit/risk ratio is considered to be negative for pipemidic acid, nalidixic acid, flumequine and cinoxacin. Their marketing authorizations in the EU should be suspended. The benefit/risk ratio remains positive for ciprofloxacin, levofloxacin, perfloxacin, moxifloxacin, ofloxacin, norfloxacin, lomefloxacin, prulifloxacin and rufloxacin.

**Conclusion**

The conducted analysis showed that fluoroquinolones should not be used as a first line therapy for infections, especially when the condition is self-limiting or uncomplicated. Quinolones remain important treatment option but in cases meeting certain criteria and in case of quinolones sensitive pathogen. Overall safety profile of the quinolones pharmacological class is considered to be well known, with the majority of frequent ADRs being non serious. However, recent assessment of safety data shows that rare ADRs are linked to long duration and risk of disability. Limiting the indications for use of fluoroquinolones is an important measure to minimize risk of serious disabling ADRs and development of microbiological resistance.

**References**


Molecular docking of monoamine oxidase A with xanthones from Hypericum perforatum roots

Marija Todorovska*, Jovana Georgieva, Oliver Tusevski, Sonja Gadzovska Simic

Laboratory of Plant Cell and Tissue Culture, Institute of Biology, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University, Arhimedova 3, 1000 Skopje, Republic of North Macedonia

Introduction

Hypericum perforatum L. (St John’s wort) represents the most studied medicinal plant throughout the world due to the presence of a broad range of secondary metabolites with biological activities. Phenolic compounds, such as flavonoids naphtodianthrones, phloroglinesols and xanthones are the main bioactive metabolites commonly described for this plant (Nahrstedt and Butterweck, 2010). The most significant use of H. perforatum preparations comprises symptomatic treatments of mild-to-moderate depression and recently good perspectives emerged for major depression (Solomon et al., 2013). The in vitro studies for biological activity of various compounds from H. perforatum extracts showed the monoamine oxidase-A (MAO-A) inhibition as the possible mechanism for antidepressant effect (Thiede and Walper, 1994).

The antidepressant activity of H. perforatum has been related to the hypericins, hyperforins and flavonoids accumulated in the aerial plant parts. Recently, root extracts of H. perforatum have been recognized as the main source of xanthones with MAO-A inhibitory properties highlighting their in vitro antidepressant effects (Tusevski et al., 2018). However, the action mechanism of xanthones from H. perforatum roots for MAO-A inhibition has never been examined. In this study, molecular docking analysis was employed for the first time to elucidate molecular interactions between MAO-A with xanthones as the most abundant metabolites from H. perforatum roots.

Materials and methods

Enzyme preparation

The crystallographic structure of MAO-A enzyme (pdb: 2Z5X) in complex with harmine was downloaded from the Protein Data Bank RSCB PDB. The raw crystal structure of MAO-A was prepared with AutoDock Tools 4.2 where all water molecules, ligands and co-factors were removed, while Kollman united-atom partial charges for neutralization of the enzyme were added, as well as non-polar hydrogens were merged. All hydrogen atoms of MAO-A were further optimized by using the MolProbity application to generate a correct hydrogen bond network. This enzyme structure was saved in pdbqt format in AutoDock tools 4.2.

Ligand preparation

Mangiferin and γ-mangostin were selected as the most representative ligands for molecular docking study due to their abundance in H. perforatum roots (Tusevski et al., 2018). The ligand molecules were downloaded from PubChem database. Atomic charge and potential of the ligands were computed with VEGA ZZ program (3.1.2) using TRIPOS force field along with Gasteiger charges. The prepared

* todorovskabt96@gmail.com
Molecular docking

AutoDock 4.2 software package was used to predict the molecular interactions between the representative ligands and the MAO-A receptor by using the Lamarckian Genetic Algorithm. Standard docking protocol for rigid protein and flexible ligands was implemented with 10 independent runs per ligand. AutoGrid 4.2 program was used to calculate grid maps of 60x60x60 with 0.375 Å distance between grid points. The best ligand binding conformation was selected according to the lowest binding energy (kcal·mol⁻¹), as well the type of interaction between the ligand atoms and enzyme amino acid residues. The best docking results were analyzed and visualized using the Discovery Studio Visualizer 16.1 (Accelrys, San Diego, CA, USA).

Results and Discussion

The docking data for MAO-A showed that γ-mangostin and mangiferin exhibited different binding energies towards the active site of the enzyme. Among two tested xanthones, γ-mangostin showed the most favourable interaction into MAO-A pocket that was represented with the lowest binding energy (-9.97 kcal·mol⁻¹). In this context, γ-mangostin was found as the most prominent MAO-A inhibitor due to the formation of hydrogen bonds of OH groups at C3 and C6 position for effective MAO-A activity (Ji and Zhang, 2006). Additionally, γ-mangostin-aglycone complex was stabilized through hydrophobic interactions with amino acid residues Phe 208 (π–π T-shaped), Tyr 407 and Tyr 444 (π–π stacked), as well the cofactor FAD (π–σ–pi T-shaped). Even that mangiferin is weaker MAO-A inhibitor, its high concentration in H. perforatum root extracts could additionally contributed to the antidepressant effects.

Conclusion

Computational approach performed in the present study highlighted the action mechanism of xanthones from H. perforatum roots for MAO-A inhibition. Molecular docking data revealed that γ-mangostin and mangiferin are promising antidepressant compounds due to their capacity for establishment of hydrogen binding and hydrophobic interactions with MAO-A active site. This study provides pivotal evidence for selecting xanthones from H. perforatum roots as potential compounds for prevention and treatment of depression.

References


Leukotriene receptor antagonist (LTRA) added to regular preventive therapy: inhaled corticosteroids and long-acting beta agonists (ICS/LABA) in patients with severe uncontrolled asthma

Elena Jovanovska-Janeva¹*, Dejan Dokic¹, Biserka Kaeva¹, Gorica Breskovska¹, Zlatica Goseva¹, Zoran Arsovski¹, Olivera Krstic Naksowska¹, Dejan Trajkov², Magdalena Dimitrova Genadieva³

¹PHI University Clinic of Pulmonology and Allergy, Majka Tereza 47, 1000 Skopje, North Macedonia
²Institute of Immunobiology and Human Genetics, Majka Tereza 47, 1000 Skopje, North Macedonia
³PHI University Clinic of Gastroenterohepatology, Majka Tereza 47, 1000 Skopje, North Macedonia

Introduction

Asthma is a worldwide problem and also one of the most common chronic diseases worldwide - 334 million patients suffered from asthma. It is estimated that number is expected to reach 400 million by 2025. Prevalence was increasing in many countries, especially in children - 6% and in adult 10%. There are 250,000 deaths annually (GINA, 2019). In the Republic of North Macedonia 100,000 or 5% of the population suffers from asthma (Cvetanov, 2006).

Asthma is a heterogeneous disease, characterized by chronic airway inflammation of the airways in which many cells play a role, in particular mast cells, eosinophils, and T lymphocytes. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation that is at least partly reversible either spontaneously or with treatment (GINA, 2019).

In this chronic inflammation are involved more than 100 mediators and the interleukins take central place in this inflammation. The interleukins are cytokines that stimulate the proliferation and differentiation of immune cells. T cells play a key role in coordinating the immune response in asthma. Th2 cells produce, IL-4, IL-5, IL-9, IL-13, GM-CSF and IL-25, IL-31, IL-33 that are responsible for chronic eosinophilic inflammation, inflammation in allergic diseases, including asthma (Hamid et al., 2003; Zhu et al., 2010).

The aim of this study was to determine the effect of combined therapy: leukotriene receptor antagonist (LTRA) added to regular preventive therapy consisted of inhaled corticosteroids and long-acting beta agonists (ICS/LABA) in patients with severe uncontrolled asthma, by analyzing of IL-5, forced expiratory volume in the first second (FEV1) and Symptom score at the beginning and after 6 months of therapy.

Materials and Methods

The study included 29 patients with severe uncontrolled asthma. They were treated with LTRA, Montelucast 10 mg/daily and combined therapy of (ICS/LABA) 500/50 mcg twice daily in duration of 6 months. In each of them were measured serum IL-
5 levels by the ELISA method before the treatment and after 6 months of therapy at the Institute of Immunobiology and Human Genetics, Faculty of Medicine, Skopje. Spirometry was done at the Clinic of Pulmonology and Allergy, Skopje. We determined FEV1 and assessed Symptom score with 5-point Likert scale of breathlessness at the beginning and after 6 months of therapy.

Inclusion Criteria included uncontrolled severe persistent asthma patients. The classification was according to the actual version of the GINA guidelines (GINA, 2019) and Guidelines of National Asthma Education Prevention Program (NAEPP, 2007). The age of the patients was 18-70 years. Exclusion criteria included: pregnancy, severe diseases of the immune, endocrine, hematological cardiac, renal, gastrointestinal and neurological system, psychiatric disorders and neoplastic diseases.

Results were statistically elaborated according to the Wilcoxon Pairs Test and T-test for Dependent Samples. The significances values were taken p<0.05 and a highly significant p<0.01.

**Results and Discussion**

The obtained results of IL-5 showed that the level of IL-5 before the start of therapy were much higher and that treatment significantly reduced their value (Z=4.64; p=0.000004). The difference in the average value of FEV1 before and after therapy was statistically significant (t=7.56; p=0.000000). There was a difference in Symptom score before and after 6 month therapy (Z=4.54; p=0.000006), pointing to significant improvement in asthma symptoms, because the patients after treatment were pleaded with much lower Symptom score.

The mechanism of action of the LTRA is based on countering the effects of cysLTs at their receptor site (CysLT1-receptor) within the airways. This results in a dual effect: suppression of the airway inflammation and mild bronchodilator properties. When is added to ICS/LABA the act complementary with improving symptoms, lung function, significant reduction in exacerbations and inflammation parameters. (Diamant and Van der Molen, 2005). Montelukast may affect eosinophilic reactions by reducing IL-5 synthesis and circulating in the asthmatic airways, and consequently to reduce airway inflammation and blood eosinophils in asthma (Hamid et al., 2003). In a smaller, open label study of 313 patients on combination therapy (ICS/LABA) they observed improvement in asthma symptoms and pulmonary function with add-on LTRA therapy after 2 months of therapy (Dupont et al., 2005).

**Conclusion**

Combination therapy with ICS/LABA represents the gold standard in the treatment of severe uncontrolled asthma and it is safe and effective treatment. However, not all patients have well control of asthma. In such patients, there is a need for additional add-on therapy, such as treatment with LTRA. Combination ICS/LABA improve symptoms and lung function, and the addition of LTRA offers additional suppression of airway inflammation, as confirmed in our study. Future studies should confirm this option.

**References**


Freeze-drying of nanostructured lipid carriers loaded with Salvia off. extract for Alzheimer’s disease treatment

Iskra Karakash*, Jovana Vasileska*, Dushko Shalabalija, Ljubica Mihailova, Marija Glavas Dodov, Renata Slaveska Raicki, Maja Simonoska Crcarevska

Institute of Pharmaceutical Technology and Center of pharmaceutical nanotechnology, Faculty of Pharmacy, Ss. Cyril & Methodius University, Majka Tereza 47, 1000 Skopje, R. N. Macedonia

Introduction

NLC (nanostructured lipid carriers) are matrix type colloidal drug carriers composed of a mixture of solid (fat) and liquid (oil) phase. Compared to solid lipid nanoparticles NLCs are characterized by higher drug loading capacity, minimized drug expulsion during storage and improved stability as a final product. Commonly used methods for their preparation are high pressure homogenization, o/w microemulsion, solvent emulsification-evaporation or –diffusion, water-in-oil-in-water double emulsion (w/o/w) as well as high shear homogenization and ultrasonication (Üner, 2006). In all cases they are produced as water dispersion and in order to extend their stability and prolong shelf-life the water should be removed using some of available techniques such as freeze-drying, spray-drying etc.

The aim of this paper was to stabilize previously prepared NLC loaded with freeze-dried methanol extract Salvia off. Extract (FSE) for Alzheimer’s disease treatment (Taneska et al., 2018) by freeze-drying. Several cryoprotectants in different concentration were used and their influence upon particle size and particle size distribution of prepared NLC-FSE was evaluated.

Materials and methods

Preparation

The NLC-FSEs were prepared using the solvent evaporation method. The lipid phase was prepared as follows: FSE (0.025 g) was dissolved in ethanol 96% (Alkaloid, Macedonia) (10 g) using ultra sonic bath for 10 min and subsequently phospholipon 90H (kindly donated by Phospholipid, Germany) (0.1 g) and oleic acid (Sigma-Aldrich, Germany) (0.065 g) were added. The aqueous phase was composed of 0.045 g poloxamer 407 (BASF, Germany), 0.3 g tween 80 (Merck, Germany) and 8.805 g of distilled water. Lipid phase was dropwise added to aqueous phase under constant magnetic stirring (68 °C, 500 rpm; IKA, Germany). Emulsion was stirred for ~2h under previous conditions until complete evaporation of ethanol and formation of NLC dispersion. The dispersion was cooled down to room temperature by magnetic stirring (300 rpm, 25 °C; IKA, Germany) and left over night on 2-8 °C for recrystallization of the lipid phase.

Freeze-drying

Volume of 0.5 mL of certain cryoprotectant solution with adequate concentration was added to NLC-FSE formulation (2 g) and gently shaked for 1 min. 16 different samples were prepared using 4

* iskra.karakash@gmail.com; * vasileskajovana@gmail.com
cryoprotectants (mannitol, sorbitol trehalose and saccharose) each in four different quantities (total lipids (solid+liquid):cryoprotectant ratio of 1:1, 1:2, 1:4 and 1:6). Samples were frozen at -20 °C for 2 h and then transferred at -80 °C for 22 h. Freeze drying was conducted at -47 °C 0.05 mBar, for 24 h (Labconco, USA). For comparison, NLC-FSE without cryoprotectant was freeze-dried at the same conditions.

Particle size and distribution determination

The freeze-dried samples (30 mg) were reconstituted with 0.9% NaCl (50 mL) under magnetic stirring (15 min, 25 °C, 300 rpm; IKA, Germany). The particle size and particle size distribution were measured by laser diffractometry (Mastersizer 2000, Hydro 2000S, UK) using previously validated method (Taneska et al., 2018). Results are expressed as particle size (D50±SD) and distribution (Span±SD). D50 and Span factor of NLC-FSE particles before freeze-drying were also determined.

Results

D50 and Span factor value of NLC-FSE before freeze-drying were 149.1±7.25 nm and 2.59±1.3, and in freeze-dried sample without cryoprotectant they were 490.9±8.02 nm and 85.27±7.93, accordingly. The higher values for D50 and Span of reconstituted freeze-dried sample could be explained by absence of cryoprotectant thus resulting in the formation of agglomerates, most likely due to disruption of the phospholipid layer on the particle surface during the freeze-drying. When sugar alcohols were used as cryoprotectants the lowest observed values for D50 and Span were determined in 1:2 ratio (338.8±4.55 nm and 5.27±0.36) for manitol and 1:1 ratio for sorbitol (157.7±5.87 nm and 5.77±0.78), subsequently. In the case of trehalose and saccharose as cryoprotectants best results were obtained for 1:2 ratio with D50 of 141.9±2.23 nm and Span of 4.14±0.07 and 155.6±1.26 nm and 4.91±0.08, accordingly.

Trehalose and sucrose in the lyophilization process form a glass matrix that represents a physical barrier between particles, reduces diffusion and molecular mobility (Abdelwahed et al., 2006). Results indicated that trehalose is more effective cryoprotectant than saccharose most likely related to the water molecule displacement due to the hydrogen bonding between the phospholipid phosphate groups and the hydroxyl groups of trehalose thus resulting with the complexation between trehalose and phospholipid and favoring the stability of the lamellar structure on the particle surface. On the other hand, sucrose does not interact with phospholipid groups and is unable to replace water molecules from the surface of the nanoparticles as in trehalose. The effect of sucrose on the surface of the nanoparticles is most likely of a colligative nature and the extraction of water molecules from the particle surface occurs by an osmotic route (Crowe et al., 2007).

Conclusion

NLC-FSE were prepared and freeze-dried using manitol, sorbitol, trehalose and saccharose as stabilizers (cryoprotectants) in different concentrations. NLC-FSE sample freeze-dried with total lipids (solid+liquid): trehalose ratio of 1:2 showed best results related to D50 and Span after reconstitution.

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Evaluation of the *in vitro* bee venom release and skin absorption from bioadhesive gel formulation

Angela Mircevska¹, Tamara Ivanoska¹, Lejla Mutapcic¹,², Dushko Shalabalija¹, Ljubica Mihailova¹, Maja S. Crcarevska¹, Metodija Trajchev³, Dimitar Nakov³, Marija Glavas Dodov¹*

¹Faculty of Pharmacy, UKIM-Skopje, Majka Tereza 47, 1000 Skopje, N. Macedonia  
²Faculty of Pharmacy, UNTZ, Univerzitetska 8 Tuzla, BiH  
³Department of Animal Biotechnology, Faculty of Agricultural Science and Food, UKIM- Skopje, Blvd. Aleksandar Makedonski bb, 1000 Skopje, N. Macedonia

Introduction

Topical and transdermal drug delivery are one of the most suitable alternative, non-invasive routes for administration of drugs in clinical practice mainly due to the increased patient compliance and reduced systemic drug side effects. Many drug products applied to the skin surface may penetrate to some extent into the skin layers, where their effects are expected, as for example, topical formulations for the treatment of different local skin disorders. Also, significant concentrations of drug could be absorbed by the body regions close to the site of delivery, where regional effects are expected, for e.g., in the muscles, local blood vessels and articulations (Ruela et al., 2016).

Arthritis is a systemic, autoimmune disease characterized by inflammation of joints. Inflammatory cytokines cause activation of the macrophages which leads to swelling of joints, damage to cartilage, bone erosion, functional impairment and stiffness (Mohanty et al., 2019). Bee venom (BV) contains a variety of peptides, including melittin, apamin, adolapin, the mast-cell degranulating peptide, enzymes (phospholipase [PL] A2), biologically active amines (histamine and epinephrine) and nonpeptide components with anti-inflammatory, anti-arthritis, anticoagulant, antimicrobial, anticancer and anti-nociceptive properties. Melittin, a major peptide component of BV shown to have anti-inflammatory and anti-arthritis properties and inhibitory activity on nuclear factor kappaB which is involved in the synthesis of inflammatory mediators and may be essential for the treatment of arthritis using BV (Son et al., 2007).

The aim of this study was to evaluate the stability of crude BV as an active ingredient, as well as to evaluate the *in vitro* release and skin absorption of BV from a designed topical gel formulation.

Materials and methods

BV sample was supplied from Kozarac, BiH, during July 2019. BV samples were stored at 25 °C and 60% RH, 2-8 °C and -20 °C for stability period of 3 months. The stability of BV was determined by a modified HPLC method (Rybak-Chmielewska and Szczésna, 2004) using melittin (Sigma, USA) as an external standard (Agilent Technologies 1200 Series; Restek Ultra C18 column; gradient elution
with 0.1% trifluoroacetic acid (TFA) in water (mobile phase A) and 0.1% TFA in acetonitrile-water 80:20 (mobile phase B)), flow rate of 2.5 mL/min, 20 µL injection volume, λ of 220 nm.

The gel was prepared by dissolving 1.75% of chitosan (CTS, low-molecular weight; Sigma–Aldrich, USA) in 1% of lactic acid solution with 0.5% of poloxamer 407 (Pluronic F127, BASF Chemtrade GmbH, Germany), 5% of propylene glycol (Alkaloid, N. Macedonia), 0.2% of potassium sorbate (Apac Chem. Corp., USA) and 0.3% m/m of BV (300 rpm, ambient temp.; Variomag, Germany). In vitro BV release from the prepared gel (1.5 g) was performed using membrane diffusion cells (MEMBRA-CELL dialysis tubing; Serva Feinbiochemica GmbH, Germany) (32±0.5 °C, 15 mL of distilled water as a dissolution medium, 300 rpm). At predetermined time intervals (after 1, 2, 4, 6, 8 and 22 h) aliquots were taken and analyzed by HPLC (n=3). In vitro permeation studies were performed by using pig skin obtained from local slaughterhouse (dermatomed, stored at -20 °C).

Before the experiment, the skin was thawed in 0.9% NaCl sol. at 37 °C and rinsed twice to remove any adherent blood or other material from the surface. The test was carried out under identical conditions as described for in vitro release studies with the difference of pig skin between donor and receptor compartment (n=2).

Results and discussion

Freshly obtained BV sample contained 43.54% of melittin. Stability studies of crude BV showed that the sample stored at 2-8 °C was the most stable one (~93% of the initial amount) during the period of 3 months. Samples stored at -20 °C and at 25 °C/60% RH for 3 month stability period showed 78.2% and 77.1% of the initial BV amount, respectively. Therefore, BV sample which was stored in refrigerator was used for further studies.

During the period of 22 h, ~90% of BV was released from the prepared gel, following the Peppas-Sahlin kinetic model. The skin absorption studies and penetration of BV trough pig skin was found to be 77.1% for 22 h and best fitted to the Peppas-Sahlin kinetic model with Tlag of 0.6 h. The steady state diffusion flux (Jss) value was 0.0319 mg/cm²/h and the permeability coefficient (Kp) was 0.1063 mg/cm²/h. Compared to in vitro BV release, the permeation was slower, most likely related to the time required for release, penetration and participation of BV through different skin layers before it reaches the acceptor compartment. High correlation coefficient (r=0.97) was found between the data from release and skin permeation studies of BV from the prepared gel.

Conclusion

According to the results obtained from this study it could be concluded that freshly obtained BV could be stored at a temperature of 2-8 °C for prolonged time period. Based from in vitro performed studies, gel formulation prepared with 1.75% CTS and 0.5% PL could be a promising candidate for efficient topical delivery/treatment of BV for arthritis. Further clinical studies should be conducted.

References


Pharmaceutical care - a patient right to health care service in R.N. Macedonia

Iskra Jordanovska*, Katerina Anchevska-Netkovska,
Aleksandra Kapedanovska Nestorovska, Aleksandra Grozdanova

Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, N. Macedonia

Introduction

The healthcare system is an exceptionally important segment of the social country which needs to provide constant quality and continuous health protection as stated in the Health Care Law of Republic of North Macedonia “Every citizen has the right to obtain the health protection by respecting the highest possible standard of human rights and values, that means having the right to physical and psychological integrity and security of his/her personality, as well as to his/her moral, cultural, religious and philosophical beliefs” (Law on Health Protection, 2012).

Reaching the highest attainable standard of health, it’s not only a primarily patient right but a fundamental right of human beings and medicines are considered as priorities of attaining this right (WHO). In this context, not only providing medicines, but also their rational use is indispensable responsibility of the health care system. Despite the reorganization of health care and social services, the growing expectations of society demands for services that are more focused on the individual patient, a higher quality of health care within the community and a more effective patient participation in decisions. A multi-disciplinary approach is needed to develop, implement and evaluate the interventions to promote a more rational use of medicines.

Pharmaceutical care: framework for redefining the pharmaceutical practice

Pharmaceutical care is a philosophy of practice in which the patient needs, not the drug, are the focal point of the pharmacist’s actions-attitudes, behaviors, commitments, concerns, ethics, functions, knowledge, responsibilities and skills intended to provide rational pharmacotherapy that will improve treatment outcomes and thus patient health and quality of life. Pharmaceutical care as a patient-centered, outcome oriented practice, strives to provide safe, efficient, evidence-based and cost-effective drug treatment individualized to patient needs according to the “5 Rights” concept - the right drug to the right patient, in the right dose and right route of administration, at the right time. Pharmaceutical Care recognizes the pharmacist as a health care provider who actively participates in the prevention of illness and the promotion of health of the patients, along with other responsible members of the health care team. Pharmaceutical Care practitioner cannot replace the physician, the dispensing pharmacist, nurse or any other health care provider and vice versa. Therefore, the hierarchy driven relationship has to be abandoned and all health care workers in the multidisciplinary team have to accept each other as a partners in achieving the best for the patient (Dalton and Byrne, 2017; EDQM, 2012).

* ijordanovska@yahoo.com
Pharmaceutical care services: contributing to improved healthcare system

Pharmaceutical care services, along with Public health interventions and effective medicines supply management, are key components of the rational use of drugs. Common types of irrational use include failure to prescribe, dispense, and use medicines as per guidelines, use too many medicines, inappropriate use of antibacterials, overuse where not required, underuse where required, inadequate use for chronic diseases, self-medication and use of expensive low-efficacy, low safety drugs. Published evidence shows that implementation of Pharmaceutical care services (eg. Medication Reconciliation, Adherence and Knowledge assessments, Medication Optimization, Patient Counseling) effectively prevents the irrational drug use resulting in decreased rate of morbidity, mortality, adverse drug reactions, antimicrobial resistance and financial loss (Dalton and Byrne, 2017).

Pharmaceutical care – focus on patient rights to quality health care

Pharmaceutical care ethics is the core principal in the set of pharmacist’s actions that puts patients' rights first: the patient has to have all of the information about medicines he uses; the patient has to be actively involved in making health care decision and has to articulate the desired outcome. Thus, the impact of implementation this concept, directly corresponds on the rate of patient's rights accomplishment. At the same time, patients have responsibilities too, such as being reasonable and polite, using the medicines with awareness and communicate any problem that may arise with their medication during the therapy (Code of Ethics for Pharmacists in the Republic of North Macedonia; Law on Protection of Patients’ Rights, 2008).

Implementation and regulation of Pharmaceutical care services

Regulation, as part of the public policies that guarantee health safety, is an important and essential element for the provision of quality services and products. It is defined as the capacity to generate new laws and regulations aimed at improving the health of the population. Actually, it represents the institutional capacity to develop the regulatory framework to protect public health. This function should be outlined in the national health care and pharmaceutical policies. The concept of pharmaceutical care has been well defined and broadly implemented in developed health care systems - hospitals and community pharmacy settings both. Conversely, its implementation in developing countries, as R.N. Macedonia is still theoretical and practically nonexistent with no regulation that allows the attainment of contracted pharmaceutical care services. The pharmacy is not considered as a location of “health care services” delivery, but only as a location of “retail and dispensing drugs service”.

Conclusion

Traditional concept of health care in R.N. Macedonia has already reached its limit, being necessarily to transform it, in line with the time and needs of patients nowadays. The clinical, humanistic and economic benefit of implementation of Pharmaceutical care as a patient right to a quality health care service at all levels of the health care systems in the developed countries is evident. In our country, implementation of pharmaceutical care services faces significant barriers and many challenges. A change in orientation of the pharmaceutical services with a new pharmaceutical care functions and responsibilities requires a development of a totally new regulatory framework or a huge change in the existing ones.

References

The influence of surfactants on the content of clindamycin phosphate in macrogol based compounded vagitories

Vesna Savić¹*, Slavica Sunarić¹, Jelena Živković¹, Milica Martinović¹, Ivana Nešić¹, Ivana Gajić²

¹Faculty of Medicine, Blvd. Dr Zorana Dijindjica 81, 18108 Nis, Republic of Serbia
²Faculty of Technology, Blvd. Oslobodenja 124, 16000 Leskovac, Republic of Serbia

Introduction

Vaginal route is often used for administration of different types of drugs (Đurić, 2004). Ideal vaginal preparation should be easy and painless to use, economical, widely available and safe for long-term use (Neves and Bahia, 2006). Vagitories are spherical or conical dosage forms intended for vaginal application. They consist of active substance dissolved or uniformly dispersed in a suitable, non-irritating base (Ph. Jug. V). Depending on whether vagitory bases are oleaginous or hydrophilic, they melt at body temperature or dissolve in body fluids (Krajšnik et al., 2013). Surfactants are added to enable emulsification of liquid components and better wetting of dispersed particles which can lead to improving of the release process and increasing of the spread capacity per application (Đurić, 2004). Clindamycin is a bacteriostatic lincosamide active against G-positive aerobes and a wide range of anaerobes, and is often used in treatment of bacterial vaginosis. (Sweetman, 2009).

The aim of this study was to make macrogol based vagitories, with 120 mg of clindamycin phosphate incorporated in each of vagitory, with or without nonionic surfactant (Tween 80), using compounding method and to determine the content of the active substance (clindamycin phosphate) in the prepared vagitories.

Materials and methods

Formulation and preparation of vagitories

Six macrogol-based clindamycin phosphate vagitories were made when 8.21 g of liquid Macrogol 400 (Defond Chemical, China) and 5.48 g of solid Macrogol 6000 (Defond Chemical, China), were melted in patena using laboratory water bath. Melted base was then gradually added to patena with 0.72 g clindamycin phosphate (Sigma Aldrich, Darmstadt, Germany), homogenously stirred and later poured into metal molds, lubricated with liquid paraffin (Avena Lab - Farmadria, Vršac, Serbia).

After releasing vagitories from the molds, each vagitory was measured. Vagitories with Tween 80 (Avena Lab - Farmadria, Vršac, Serbia) were made the same way, except that in addition to 7.78 g of macrogol 400 and 5.18 g of macrogol 6000, also 0.72 g of Tween 80 was melted.

Method of determining the content of clindamycin phosphate in pessaries

High performance liquid chromatography (HPLC) on chromatography system Agilent 1200 Series Diode Array and Multiple Wavelength detector (Agilent Technologies, USA) was used for determining the content of clindamycin phosphate in vagitories. ZORBAX Eclipse Plus C8 column (3.0 x 150 mm; 3.5 µm particle size) (Agilent) was used (temperature 40 °C). Mobile phase (flow: 0.8 mL/min) consisted of acetonitrile:phosphate buffer =

* vsavic203@yahoo.com
20:80 (pH=2.5). Injected volume of samples was 10 µL. Detection wavelength was 210 nm (Stanković et al., 2013). Tested samples were made by dissolving the entire vagitory in 50 mL of phosphate buffer. Afterwards, 0.83 mL of that solution (corresponding to the content of 2 mg clindamycin phosphate) was taken using an automatic pipette and transferred to a normal 25 mL vessel supplemented with phosphate buffer (pH=2.5). Obtained concentration of samples was 80 µg/mL. Basic standard solution of clindamycin phosphate was made by dissolving of 10 mg of the substance in a normal 10 mL vessel in phosphate buffer (pH=2.5). The concentration of such solution was 1000 µg/mL. The working standards of clindamycin phosphate used to record the chromatogram and obtain the reference standard curve were made by diluting of the basic standard solution with phosphate buffer (pH=2.5). In this way, 5 standard solutions of the following concentrations were made: 40 µg/mL, 60 µg/mL, 80 µg/mL, 100 µg/mL and 120 µg/mL.

Results and discussion

After compounding of vagitories, each vagitory was removed from the mold and measured. Average mass of macrogol-based clindamycin phosphate vagitories was 2.39 g while average mass of the ones with Tween 80 was 2.38 g, so mass variations were within limits prescribed by Ph Jug V (5% from expected value – 2.4 g)

For calculating the content of clindamycin phosphate in vagitories, equation of calibration curve was used (y=2.3691x+4.392, R²=0.9999).

In order to determine influence of base on active substance content, the chromatograms of placebo, standard and samples were recorded. A placebo was made solely of excipient (macrogols) The method proved to be selective because no peaks appeared on the placebo chromatograms with the retention time of clindamycin phosphate (3.534 min).

The method also proved to be accurate since chromatograms of standard samples with well-known clindamycin phosphate concentration in macrogol basis (120 mg per vagitory) showed peak at a retention time of 3.213 min. Concentrations of clindamycin phosphate (mg/pessary) and recovery values were calculated. The recovery value (the ratio of the clindamycin phosphate concentration found in the sample and the expected clindamycin phosphate concentration) was 102.9%.

Six chromatograms each of macrogol based clindamycin phosphate vagitory samples with or without Tween 80 were recorded. The mean peak area was calculated and then the equation of calibration curve of clindamycin phosphate standard solutions was used to calculate the mean concentration of clindamycin phosphate in the macrogol pessaries. The obtained clindamycin phosphate concentration in macrogol based vagitories without Tween 80 was 115.9±8.2 (recovery value 96.6%), while the mean concentration in the pessaries with Tween 80 was 119.2±2.3 (recovery value 99.3%).

Conclusion

The results showed that the content of clindamycin phosphate was higher in macrogol vagitories with Tween 80. Nonionic surfactants help releasing of active substance from the pharmaceutical form, therefore it can be assumed that it can be one of the reasons why the content of the active substance in vagitories with Tween 80 was higher. Next research may be related to the biopharmaceutical aspect of the influence of the bases in compounding. It is recommended to consider possibility of the validation of technological manufacturing process during compounding, depending on the applied excipients and additives.

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References


Effects of formulation and sol-gel synthesis conditions on physical stability and chemical structure of organomodified silica nanoparticles: a screening study

Teodora Dimkovska¹, Beti Djurdjic², Katerina Goracinova³, Boban Mugosa⁴, Nikola Geskovski¹*

¹Institute of Pharmaceutical technology, Faculty of Pharmacy, Ss. Cyril and Methodius University, Majka Tereza 47, 1000, Skopje, N. Macedonia
²Department of Pharmacy, Faculty of Medicine, University of Montenegro, Kruševac bb, 81000 Podgorica, Montenegro
³College of Pharmacy, Qatar University, PO Box 2713, Doha, Qatar
⁴Institute of Public Health, Džona Džeksona bb, 81000 Podgorica, Montenegro

Introduction

Nanoparticles (NPs) have drawn increasing interest from every branch of medicine as potential carriers for targeted drug delivery in the optimum dosage range providing increased therapeutic efficiency, and at the same time minimizing the side effects. This is especially important in cancer chemotherapy, where patients experience severe side effects that seriously affect the quality of life. However, many factors (i.e. particle size, zeta potential, drug loading, stability, interactions with the biological milieu) influence the overall efficacy of the nanoparticulated drug carriers. Therefore, an in-depth approach is needed to design such formulation, evaluate and understand its biological behavior and predict its possible toxicity and therapeutical effects.

The aim of our study was to screen the effects of preparation procedure and formulation factors (pH, silica precursors ratio) on the physical stability (particle size, polydispersity index, zeta potential) in different relevant media, and the chemical structure of silica and organomodified silica nanoparticles.

Materials and Methods

Materials

Tetraethoxysilane (TEOS) and 3-aminopropyl triethoxysilane (APTES) were purchased from Sigma Aldrich (Germany); Ethanol (96%), Sodium hydroxide and Acetic acid were purchased from Alkaloid AD (N. Macedonia). All other reagents and chemicals used were of analytical grade.

Methods

Previously described sol-gel method was used for the preparation of the silicate nanoparticles (Djurdjic et al., 2018). The process involves hydrolysis and condensation of metal alkoxides (Si(OR)₄) such as TEOS, APTES in the presence of mineral acid (e.g., HCl) or base (e.g., NH₃) as a catalyst. Pure TEOS and TEOS/APTES NPs in the ratio of 99:1 were prepared. The physical stability of formulations was tested by diluting the corresponding sample volume of 300 μg NPs with
an appropriate medium (0.1M HCl, phosphate buffer 4.5 and 7.4) of up to 3 mL and incubating at 37 °C. Appropriate volume (100 μL) was taken 1, 3, 5 and 7 hours after the start of incubation. Particle size (PS), polydispersity index (PDI) and zeta potential (ZP) analysis were performed by dynamic light scattering using the Malvern Zetasizer Nano ZS90 (Malvern Panalytical, UK). Fourier transform infrared spectra were acquired on freeze-dried samples using Nicolet iS10 (Thermo Scientific, USA). Multivariate analysis (SIMCA 14, Umetrics, Sweden) was employed to investigate the possible effects of the formulation and process variables upon the NP chemical structure (FTIR spectra) and in vitro stability of their PS, PDI and ZP.

Results and Discussion

Categorical PCA X&Y models were employed to identify the effects of the independent variables (pH during the sol-gel synthesis of NP, silica precursors TEOS/TEOS-APTES) upon the NP’s PS, PDI, ZP stability trends and FTIR spectra. Each model contained two main components explaining (R^2X) 90.8, 98.3, 99.4 and 98.8% of the variations in PS, PDI, ZP and FTIR spectra, respectively. The predictivity coefficient (Q^2) was within satisfactory limits (0.825 – 0.986) for all models. The results revealed that the hydrodynamic diameter of the particles and their stability in pH 7.4 was mainly governed by the silica precursors (TEOS/TEOS-APTES) employed in the formulations, while no significant variations among the formulations in PS stability were observed in pH 1 and 4.5. The PDI of the formulations in all tested media was affected by both the preparation procedure and silica precursors. The stability of ZP at pH 1 was mainly influenced by the silica precursors, while the pH of the preparation procedure mainly affected the ZP stability at pH 7.4. All observed effects were probably due to the surface-oriented amino groups that govern the surface potential of the particles affecting particle growth during synthesis and stability during incubation, and the pH of the sol-gel synthesis process which also affects the silica hydrolysis rate and condensation behavior, thus resulting in noticeable effects on the PS, PDI and ZP (Wu et al., 2013). The SNV derived FTIR data revealed that the position and intensity of the bands at 1549, 1410, 1090, 902 and 770 cm\(^{-1}\) were most affected by the silica precursors used in the synthesis procedure, while the pH of the sol-gel synthesis procedure didn’t demonstrate any significant effect on the FTIR spectra. The bands at 1090 and 770 cm\(^{-1}\) correspond to the antisymmetric and symmetric stretching of the Si-O-Si species from the silica NP matrix and were blue-shifted in the pure SiO\(_2\) matrices. The bands at 1549 and 1410 cm\(^{-1}\) originate from the deformation CH2 and NH2 vibrations of the alkylamino chain, which appeared only in the APTES based formulations, while the band at 902 cm\(^{-1}\) is associated with the Si-OH and Si-O-R stretching modes (Brinker and Scherer, 2013).

Conclusion

Silica nanoparticles can be prepared by the hydrolysis reaction of TEOS and APTES in ethanol using mineral acid or base as a catalyst using the sol-gel method. In this screening study, using multivariate analysis, we have observed the effects of the silica precursors ratio and pH of the sol-gel synthesis procedure upon the stability patterns of the formulations (PS, PDI and ZP) in different biorelevant media and their surface chemical structure. The generated data pool could be used as a platform for further development and optimization of drug-loaded silica-based nanoparticles.

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References


Community pharmacists’ attitudes toward the chronic disease management in R.N. Macedonia – part II

Donka Pankov*, Nikola Geskovski, Maja Simonoska Crcarevska, Marija Glavas Dodov

Institute of pharmaceutical technology, Faculty of Pharmacy, Ss. Cyril and Methodius University, Majka Tereza 47, 1000 Skopje, N. Macedonia

Introduction

Worldwide patients tend to consult pharmacists as a first call for advice, not just for their medication therapy but also for the health problems. Community pharmacists are increasingly expected to improve disease management by aiming to improve the effective drug therapy, reducing drug-drug interactions and drug-related side effects. Creating personal patient relationships and fostering communication can help to improve medication adherence and make chronic disease management more successful (Cheema, 2015).

Patient engagement is one of the best tools in a community pharmacist’s arsenal in handling chronic disease management. Making sure patients are involved in and knowledgeable about their health is vital when addressing a chronic disease. Namely, when patients are fully engaged in their care, they are more likely to maintain treatment plans, follow their health, and ask questions about their illness or life-style protocols (Krist et al., 2020).

Therefore, the aim of the present study was to investigate community pharmacists’ attitudes, perceptions, practice and perceived barriers in ensuring the proper pharmaceutical care for patients with chronic diseases in the Republic of North Macedonia (R.N.M).

Materials and methods

For the purpose of this survey, a structured pre-tested (Pankov, 2016) questionnaire was used. The questionnaire included 13 items related to the community pharmacists’ attitudes and perceptions in everyday practice with chronic disease patients as well as their knowledge for chronic disease management.

The responses were collected between September 1st 2016 and February 28th, 2017.

Obtained data were tabulated using Microsoft Excel® (Microsoft Corp. Redmond, WA, USA), computed and consequently evaluated using statistical software STAT-GRAPHICS Centurion XVI evaluation (Stat Point technologies Inc., USA).

Results and discussion

The study is based on responds of 310 community pharmacists of which 82.58% were females. The average age was 38.83±9.8 years. Majority of pharmacists, 57.42% think that patients with chronic diseases are not well educated to the level where they can successfully manage their disease, while 36.13% think that patients are educated, but not all of them.

A vast majority of 80% agreed that with the extension of pharmaceutical care and education of patients with chronic diseases, use of prescribed...
drugs will be more clarified to the patients and their compliance will be improved. Similar percentage of pharmacists (~83%) responded affirmatively to the possibility of augmentation of health care services and their active engagement in the care and prevention of patients with chronic diseases.

Nearly 75% of respondents think that through the active involvement of pharmacists in the education of chronic patients how to use correctly medical devices (inhalers, insulin pens, or medical devices to control blood pressure, etc.) patients will be able to use them properly and will successfully manage their therapy as well as that the outcome of the therapy will improve.

Almost half of the pharmacists (57.42%) agreed that the pharmacist, as the most accessible health worker, can be actively involved in the community pharmacy setting in measuring blood pressure, glucose and blood cholesterol, etc. However, 25.81% do not agree with these activities as they think that there are no conditions in the setting and 16.77% think that they should not be conducted by a pharmacist. Also, 77.42% believe that the pharmacists can contribute to the prevention of chronic diseases through life-changing services such as weight regulation, smoking cessation and alcohol consumption, assessment of risk factors for certain chronic diseases, while 9.35% do not agree with previous.

84.52% think it is necessary for pharmacists to inform medical doctors if they are carrying out additional pharmaceutical care in order to achieve better collaboration.

Faculty of pharmacy, UKIM - Skopje and University clinics are most trusted among pharmacists (80.32%) when it comes to continuous education related to pharmaceutical care in the prevention and care of patients with chronic diseases. They are followed by pharmaceutical companies (12.9%) and internet education 6.77%.

47.42% noted that their pharmacy can provide place/space for the chronic disease patients’ education/consultation, while 21.61% responded that it is not possible. In the context, majority (77.1%) responded that are interested in providing additional pharmaceutical care to patients with chronic diseases if the adequate conditions are fulfilled and only 3.87% are not interested. Nevertheless, 48.06% think that they have appropriate level of knowledge for the provision of education/pharmaceutical care of patients with chronic disease and similar percentage (48.06%) stated that will need additional training. Merely, 4.52% and 0.97% noted that they do not know or are not ready, consequently.

Immense majority (83.23%) think that additional pharmaceutical care should be valued by extra reimbursement and only 11.61% think that it should be in the frame of usual pharmaceutical care i.e. without any supplementary reimbursement.

Opinion of 17.48% of respondents is that by additional pharmaceutical care in general health costs will be reduced as a result of prevention/delay of chronic diseases and their complications, 6.45% think that health services by other health professionals will be reduced and thus savings will be produced and majority - 69.33% agree with both. Only, 6.45% do not agree with either.

**Conclusion**

The results of the conducted study pointed that community pharmacists in the Republic of North Macedonia possess knowledge, possibility and willingness to implement additional pharmaceutical care for patients with chronic disease in the everyday community pharmacy practice. Also, they stated that such activities should be valued by added reimbursement. By provision of supplementary pharmaceutical care for patients with chronic diseases they agree that patient therapy management and compliance will be significantly improved and thus the outcome of the overall medical treatment will be more effective.

**References**


Short communication

Prosthodontic practice challenges post COVID-19 outbreak

Borjan Naumovski¹*, Marjan Petkov², Svetlana Gacheva Cvetkova², Sanja Panchevska²

¹University Dental Clinical Centre St. Panteleimon- Skopje, Str. Majka Tereza 47, 1000 Skopje, RN Macedonia
²Dental Faculty, Ss. Cyril and Methodius University, Str. Majka Tereza 47, 1000 Skopje, RN Macedonia

Introduction

The novel Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) has rapidly developed into a worldwide pandemic and become a public health crisis of global concern. Due to the widespread transmission and dental practice specificities including the generation of aerosols throughout dental procedures, dental healthcare professionals and patients are facing increased risk of SARS-CoV-2 infection and infection spread.

Oral health care has its own specificities, especially taking into account dental procedures performed on elderly patients above 65 years of age with additional co-morbidities such as: diabetes, pulmonary or cardiovascular disabilities, making them more vulnerable to SARS-CoV-2 infection and worse prognosis. Geriatric patients most commonly need prosthodontics treatment including removable, complete or partial dentures (RPDs). Prosthodontists are facing significant challenges in controlling SARS-CoV-2 infection as their great exposure to aerosols and blood during tooth preparation for crown and bridge manufacturing, and to high concentration of abundant saliva in trays and dentures while producing removable dentures as well. The process of removable (partial or complete) and fixed dentures (dental crowns or bridges) manufacturing includes not only the prosthodontist and assistant, but lab technician and lab supervisor as well. As a result, the prosthodontic treatment is more complex, with more professionals involved, thus increasing the possibility for SARS-CoV-2 contamination and infection spread. (Sekhsaria et al., 2020) The aim of this study was to emphasize the influence of SARS-CoV-2 in changes of prosthodontic clinical practice.

Transmission routes of COVID-19 in dental clinics

Prosthodontic procedures are associated with risk of COVID-19 infection due to the face-to-face contact, common exposure to blood, saliva and instrument handling. Dental specialists and patients could be exposed to pathogenic microorganisms that infect oral cavity and respiratory tract. These microorganisms could be suspended in the air for long periods and could be transmitted through airborne spread. Moreover, dentists could be exposed to direct contact with oral fluids, blood, or contact with nasal, mucosal or conjunctival droplets and aerosols from infected patients due to short distance, especially if the dentist is working without proper self-protection, e.g. not wearing a mask or contact with contaminated instruments or surfaces. It has

* naumovskiborjan@gmail.com
been confirmed that salivary gland epithelial cells can potentially be infected by SARS-CoV-2 virus and become a key source of the virus in saliva. The latest research data suggest that some virus strains could be present in the saliva up to 29 days (Peng et al., 2020). The working surface area could be contaminated for prolonged period with small particles of droplets and aerosols mixed with potentially infected patient’s saliva or blood spread with high-speed turbine that works with running water.

Essential in obtaining, COVID-19-safe and sterile working environment in prosthodontic office is contentious disinfection, as well as wearing self-protection by the dental specialist. Critical step in this procedure is appropriate education for the patient, advising especially the elderly patients to disinfect the dentures at home regularly, as well as before sending them to the clinic due to ill-fitting of partial denture/complete denture (RPD/CD) and immediately reject denture wearing if infected with COVID-19. In order to obtain minimization of viral load in the operatory Negative ion generators and devices equipped with High Efficacy Particulate Air (HEPA) filters are recommended as they can efficiently remove particles less than 0.3 microns in size, in the surrounding air in the dental/laboratory offices. Prosthodontic specialist, nurses and dental technician, are obliged to reduce all elective treatment procedures that are associated with aerosol production and handle emergency situations only, with complete personal protection wearing special gloves, N95 mask, face shield, protective suit, head cap and shoe cover. It is recommended patients to be covered with a full length drape with their hands tucked in, a head cap and goggles. The oral mucosa could be disinfected with the Betadine solution while face skin surrounding the mouth opening could be wiped before commencing the procedure. Additional mandatory pre-procedural rinse is inevitable for microbial reduction in oral cavity, but instead of 1% hydrogen peroxide or 0.2% povidone iodine should be used as COVID-19 is proven as oxidation susceptible virus. These emergency situations are correlated with lost or fractured partial denture/complete denture RPD/CD by patients and the standard laboratory protocol should be followed with proper disinfection of the impression and casts. (Alharbi et al., 2020). In urgent situations associated with dislocated crown, fixed partial dentures (FPD) or implant prosthesis, patients are advised to safely keep the prosthodontics material in a box with butadiene solution. The dental appointment is recommended only after confirmation that the patient is healthy and COVID-19 negative.

Conclusion

No universal or national protocols and guidelines have been accepted or implemented for dental care provision for suspected or active virus infractions during epidemic, pandemic or global disaster. As a result of such deficiencies, prosthodontics care has been substantially declined or even completely forbidden for a period of 3 months in our country and in many COVID-19 affected countries.

The progressive spread of SARS-CoV-2 virus has made substantial changes in everyday prosthodontic clinical practice in order to obtain safe environment for patients and dental professionals. Number of precautions and safety measures have been recommended and implemented after COVID-19 became pandemic. According to the relevant recommendations, all patients should be considered as potentially infected by COVID-19 and prosthodontics team must use complete personal protection and follow strict procedures, obtaining safe working operations.

References

Dental prosthetic materials and adverse drug reactions in everyday prosthodontic practice

Borjan Naumovski1*, Vesna Jurukovska Shotarovska2, Aneta Mijoska2, Sasho Elenchevski2

1University Dental Clinical Centre St. Panteleimon- Skopje, Str. Majka Tereza 47, 1000 Skopje, RN Macedonia
2Dental Faculty, University "Ss. Cyril and Methodius", Skopje, RN Macedonia

Introduction

Prosthodontics is a dental medicine branch that resurrects and restores the missing teeth to reestablished masticatory and phonetic functions, appearance and health of the patient in order to maintain the natural state of stomatognathic system. It embraces different dental restorations, including complete dentures (CDs), fixed partial dentures (FPDs), removable partial dentures (RPDs) or implant-assisted prostheses. Dental alloys, polymer materials, acrylic resins, ceramics, cements, sealers, etchants, solutions for oral disinfection, waxes and elastomeric impressions are the most commonly used materials in prosthodontic clinical practice. During the dental procedure, patients and the medical staff are exposed to potentially harmful and irritating compounds of the used dental materials. The degradation of these biomaterials may cause various health problems and side effects. (Asal et al., 2017) The aim of this study is to evaluate the risks of adverse reactions regarding prosthodontic materials.

Materials in clinical prosthodontic

Restorative materials, impression materials and luting cements are three groups of most commonly used materials in clinical prosthodontics.

Restorative materials

Clinical prosthodontic treatment includes CD and RPD, FPD, superstructures over implants and veneers made utilizing ceramics, polymers and metal alloys.

Ceramics. Dental ceramics are made of metal oxides and half metals (K2O, CaO, MgO, Al2O3, B2O3 and SiO2).

Polymers. Polymer-based materials are used for production of veneers for crowns, FPDs, CD and RPD. Dental technicians, during the process of production of dental prosthesis, are operating with various polymers and monomers, (poly) substituted acrylic acid esters, polyvinyl esters, polyacrylic acid esters, rubber-modified polymetacrylic esters, polystyrene, polysulfones, polycarbonates and mixtures of different polymers.

Metals alloys. Most frequently used dental alloys for production of partial and fixed denture frameworks are composed of the following elements: Co (60-65%), Cr (27-30%), Mo (5-6%), Ni (≤ 0.5%, but may exceed up to 10%), Ag, Cu, Pd, Sn, Au, In, Zn and Hg. Some metals, if highly concentrated, can trigger toxic reactions in human body such as hypersensitivity, generalized and local skin reactions, reported by prosthodontic specialists worldwide.
Impression materials

There are two main groups of impression materials: elastic (hydrocolloids and elastomer) and non-elastic (gypsum products, wax materials and thermoplastic materials). Elastomers are classified into 3 groups: polymer-based elastomers, type A (addition-polymerized) and C (condensation-polymerized) silicones, polyethers and polysulfides.

Luting cements

Luting cements are used to temporarily or permanently merge crowns and fixed partial dentures (FPD) with previously prepared teeth. Water-based (glass-ionomer, zinc polycarboxylate and zinc phosphate), as well as polymer-based types of cements (polymer materials, composites, and compomers) are commonly used in everyday prosthodontic practice. (Lygre, 2002)

Adverse reactions of prosthodontic materials

Biological systems may have harmful or destructive effects on materials, recognized as biodegradation. Biodegradation of the dental materials used in the oral cavity is due to the processes of destruction and dissolution in saliva, chemical/physical destruction, attire and erosion caused by food, chewing and bacterial activity.

Alloys used in prosthetic dentistry release ions; the process is gradual, long lasting, characterized with continuous discharge of small amounts of substances. The small sized compounds released from dental restorations are absorbed in oral mucosa or the gastrointestinal tract and respiratory system. Biodegradation, initiated by the contact with saliva and biomaterials form the dental crafts, may instigate processes of hydrolysis or electrolysis, resulting in release of compounds in the oral cavity and promotion of adverse reaction for the patient.

Most dental materials are intended for long-term use and patients may be sensitized with the exposure, resulting in contact allergies, characterized with diversity in the clinical manifestations. Hypersensitivity reactions may affect the oral mucosa, with burning and pain, or appear as more objective manifestations, localized to the buccal mucosa, tongue, and lips, including stomatitis protetica, cheilitis and lichenoid reactions. Dental professionals can get hand dermatitis from using dental materials and products. Patch testing is recommended to detect contact allergies from complete and partial dentures containing the most frequently used dental materials. (Rai et al., 2014)

Hypersensitivity reactions are classified into following four different types: Type I-IV, anaphylactic antibody-mediated reactions, (most common), cytolytic or cytotoxic reactions, immune complex reactions and delayed-type hypersensitivity reaction, respectively.

Beside the vast majority of all prosthodontic dental materials available in our country and their utilization in everyday dental practice, there are no reports of adverse events associated with these materials. The main reason for this underreporting lays in the lack of awareness of the importance of adverse event reporting, lack of education program for dental specialists, technicians, nurses and patients and lack of established system for evaluation of risk/benefit ratio of dental materials.

Conclusion

Implementation of appropriate strategy to raise the awareness of the importance of adverse events reporting for dental materials used in prosthodontics, entailing educational program with workshops and practical aspects regarding materiovigilance for both dental doctors and patients, alongside with contemporary software infrastructure available on Malmed- Macedonian National Regulatory Agency, could be a successful approach for improvement of adverse event reporting in everyday dental practices. This approach has a pivotal role in obtaining effective and safe treatment and collecting inevitable information for the quality of dental materials utilized among dental health care professionals on national level.

References

Nanostructured lipid carriers as drug delivery systems for miRNA

Amina Tucak¹, Merima Sirbubalo¹, Jasmina Hadžiabdić¹, Ognjenka Rahić¹, Ivana Ruseska², Andreas Zimmer²*, Edina Vranić¹

¹University of Sarajevo, Faculty of Pharmacy, Department of Pharmaceutical Technology, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia & Herzegovina
²University of Graz, Institute of Pharmaceutical Sciences, Department of Pharmaceutical Technology and Biopharmacy, 8010 Graz, Austria

Introduction

MicroRNAs (miRNAs) represent endogenous small RNAs that post-transcriptionally regulate gene expression and, thus they are involved in the onset and progression of various diseases and conditions (Bader et al., 2010) such as for overweight and obesity. Antiadipogenic miRNA-27a is a negative regulator in fat metabolism, which inhibits adipocyte differentiation through downregulation of adipogenic marker genes (e.g. PPARγ) (Kim et al., 2010).

Reduced miRNA-27a levels are often associated with the development of obesity and, therefore, this miRNA might represent a promising candidate for miRNA mimic replacement therapy (Lin et al., 2009). However, the application of naked RNAs has shown low membrane permeability, cellular uptake, and rapid degradation in the circulation.

The present study aimed to develop a cationic, lipid-based nanoparticle system for targeting adipose tissue and delivering miRNA-27a. These systems are composed of positively charged nanostructured lipid carriers (cNLCs) and negatively charged miRNAs, which results in complex formation based on electrostatic interactions between these components.

Materials and methods

For the preparation of cNLCs, stearylamine (SA) (Sigma-Aldrich, Germany), Miglyol® 812, (Herba Chemosan Apotheker-AG, Austria), Precirol® ATO 5 (Gattefossé Deutschland GmbH, Germany), Tween® 80 (Sigma-Aldrich, Germany), Pluronic® F68 (BASF, USA), and ultra-purified water, Milli-Q® (Millipore S.A.S., France) were used. Double-stranded miRNA-27a with the sequence UUCACAGUGGCUAAGUUCCGC (Dharmacon, GE Healthcare, Austria) was purchased. Nuclease free water (VWR, Austria) was utilized to reduce the risk of nucleic acid degradation.

Production of nanostructured lipid nanocarriers

Stearylamine (0.15%; w/w), Precirol® ATO 5 (4.365%), and Miglyol® 812 (0.485%) were melted at 70 °C. The aqueous solution composed of Tween® 80 (1%) and Pluronic® F68 (1%) was simultaneously heated to the same temperature and added to the lipid phase. The coarse emulsion was obtained by mixing for 60 sec at 8000 rpm using the Ultra Turrax (IKA1-Werke GmbH & Co., Germany) and then transferred into the high-pressure homogenizer.
(Panda 2K, NS1001L, GEA Niro Soavi, Germany) operating at 800 bar and 70 °C (5 cycles).

**Formation and characterization of miRNA-cNLC complexes**

The stock solution of miRNA-27a was diluted with RNase-free water to 1.3 μM solutions. The working solutions of the cNLCs and miRNA-27a were intermixed using equal volumes and incubated at room temperature for 15 min and 5 min of sonication (Emmi-D100, EMAG Technologies, Germany) containing final miRNA concentration of 650 nM. To obtain samples suitable for the particle size (100 nM) and zeta potential (80 nM) measurements, complexes were diluted with RNase-free water. We investigated the influence on nanoparticle assembly and physicochemical properties of different mass ratios (miRNA27a: SA) ranging from 5:1 to 1:5.

Particle size, polydispersity index (PdI), and zeta potential (ZP) were determined by dynamic light scattering (DLS) and electrophoretic light scattering (ELS) with the Zetasizer Nano ZS (Malvern Instruments).

**Gel Electrophoresis**

To evaluate if there is any unbound miRNA in complexes left, E-Gel Power Snap Electrophoresis System with the E-Gel™ Power Snap Camera (Thermo Fisher Scientific Inc, Austria) was used. Briefly, 20 μL of miRNA27-a, and complexes of miRNA-27a: SA (1:1 to 1:25) were loaded into the wells of the 4% E-Gel™ agarose gel.

**Results and discussion**

The characterization of particle size by PCS revealed that the average particle size of the cNLC on the production date was 102.30±0.45 nm, and the PdI of 0.166±0.011 showed that formulation has unimodal distribution. Since the addition of SA increases the pH of formulations due to the presence of primary ammonium groups in its structure, pH was measured immediately after production (pH 8.89±0.07). The pH was thus, adjusted to around 7 to produce a full ionization of SA molecules at the interface of NLCs and, subsequently, the ZP was measured (31.73±0.46 mV).

Complexation of cNLCs with miRNA-27a in ratios from 5:1 to 1:5 led to particle sizes ranging from 238.00±3.88 nm (1:5) to 123.5±1.56 nm (5:1). However, the highest particle size (430.8±24.68 nm) and PdI of 0.354±0.056 nm were observed in a complex that contained SA and miRNAs in equal mass ratio due to the low ZP (4.09±0.09 mV) that is related to the formation of aggregates of lipid phase. ZP measurements revealed a shift from a negative value (-20.50±0.62 mV), in case of 5:1 ratio, to positive surface charges (28.50±0.48 mV), in 1:5 ratio. This result suggests that cationic cNLCs nature enables electrostatic interactions with miRNAs to form self-assembled particles. Gel electrophoresis showed that above 1:5 mass ratio, there was no presence of unbounded miRNA, suggesting the successful cNLC-miR-27a complex formation.

**Conclusion**

The physicochemical investigations using DLS, ELS, and gel electrophoresis have shown the self-assembled nature of cNLC-miRNA-27a as complexes. Therefore, future studies are planned in an in vitro cell culture model to study the anti-adipogenic effect of miRNA-27a on adipogenesis by applying our cNLC-based DDS for nucleic acids.

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**References**


