

Effect of phospholipid-polymer conjugate on physical properties of Rosuvastatin/Ezetimibe loaded lipid-polymer hybrid nanoparticles

Nadica Vanova Nakjinova^{1,2}, Nikola Jovanovikj^{1,2}, Maja Simonoska Crcarevska¹, Nikola Geskovski¹, Lina Livrinska¹, Kristina Mladenovska¹

¹Faculty of Pharmacy, Ss. Cyril and Methodius University, Majka Tereza 47, 1000, Skopje, Republic of N Macedonia

²Research and Development, Alkaloid AD, Pharmaceutical Chemical and Cosmetics Company, Aleksandar Makedonski 12, 1000 Skopje, Republic of N Macedonia

Introduction

Combination lipid-lowering therapies have proven many times more successful in reducing LDL cholesterol levels compared to monotherapies, especially when a more aggressive reduction in cholesterol is needed to help prevent atherosclerotic disease. Rosuvastatin (ROS) is one of the most potent statins and is currently widely prescribed. Even at 10 mg dose has proven more effective than other statins as atorvastatin or simvastatin at higher doses. Ezetimibe (EZE), an inhibitor of the intestinal absorption of dietary and biliary cholesterol, in combination with ROS holds the potential in achieving the desired results in a more efficient way, irrespective of the daytime of administration, although peak of hepatic 3-hydroxy-3-methyl-glutaryl-coenzyme A reductive activity and cholesterol synthesis occur at night (Chilbert et al., 2022).

In order to increase the therapeutic index of ROS and EZE combined therapy, in a previous study they were incorporated in lipid-polymer hybrid nanoparticles (LPHNPs) prepared by already described one step nanoprecipitation method (Nakjinova et al., 2022). Owing to its two-in-one structure, LPHNPs provide an excellent platform for combinatorial and targeted drug delivery in addition to superior absorption enhancing effects (Tang et al., 2020). One expects that oral combination nanoparticle therapy of ROS and EZE would have higher bioavailability at the site of action and therefore higher efficacy at lower doses with more favorable safety profile.

Among the critical material attributes that majorly regulate the physical characteristics of LPHNPs are lipids i.e. lipid type, lipid vesicle size, surface potential of the outer lipid shell and lipid-to-polymer ratio. In this paper, the effect of type and concentration of phospholipids (soybean phosphatidylcholine and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine/DSPE) used for preparation of ROS/EZE loaded LPHNPs on their physical properties was evaluated.

Materials and methods

LPHNPs were prepared from ester terminated poly (D,L-lactide/glycolide) (PLGA, 50:50, inherent viscosity 0.4 dL/g), obtained as a gift from Corbion (Netherlands), and from soybean phosphatidylcholine and DSPE-PEG2000 or non-PEGylated DSPE, obtained as gifts from Lipoid GmbH (Germany). As a stabilizer in the synthesis of drugs loaded LPHNPs, Poloxamer 188 was used, purchased from BASF (Germany). ROS (calcium salt) and EZE (free base) were purchased from DSM and Lupin Ltd. (India), respectively. Acetonitrile (ACN) was used as organic phase solvent, supplied by Merck (Germany). All other chemicals were of analytical grade.

Preparation of drugs loaded LPHNPs: ACN solution of active ingredients EZE (0.8 mg/mL) and ROS (0.5 mg/mL), and PLGA (7.5 mg/mL) was prepared and added drop-wise into the 4% w/w hydroethanolic phospholipid dispersion (1:4 phase volume ratio) pre-heated at 65 °C, under constant mixing, allowing self-assembly of

nanoparticles to occur with subsequent ACN evaporation. Seven formulations of ROS/EZE loaded LPHNPs were prepared in which concentrations of DSPE-PEG2000 in a range 0.06-0.25 mg/mL and soybean phosphatidylcholine in a range 0.28-0.66 mg/mL were varied. In a separate experiment, non-PEGylated DSPE instead of DSPE-PEG2000 was used at 0.06 mg/mL and at concentration of soybean phosphatidylcholine 0.56 mg/mL. Poloxamer 188 as a bi-functional block copolymer surfactant in 1:1 weight ratio to polymer was present in the hydroethanolic phase. The drugs loaded LPHNPs were initially vacuum filtrated through 2-3 μm pore size cellulose filter and the filtrate was subject to ultracentrifugation using 3 kDa cut off filters (Amicon®, Merck, Germany) to separate drugs loaded LPHNPs from non-encapsulated drugs.

Physical characterization of drugs loaded LPHNPs:

The hydrodynamic particle diameter (z-average), polydispersity index (PDI) and zeta potential (ZP) of ROS/EZE loaded LPNPs were determined using Zetasizer Nano ZS-100 (Malvern Instruments Ltd., UK). Encapsulation efficiency (EE) was calculated by indirect method, analyzing the untrapped drugs present in the 3 kDa resulting filtrate (HPLC method, Zorbax Eclipse XDB-C18, 80Å, 4.6 x 150 mm, 5 μm column; Agilent, USA). Drugs content (DC) was calculated based on amount of encapsulated drugs divided by total LPHNPs weight.

Results and discussion

ROS/EZE loaded LPHNs were prepared, with narrow particle size distribution (PDI 0.189-0.610), z-average from 124 to 163 nm and slightly negative to relatively neutral ZP (-2.090 to 0.443 mV). Higher EE for the hydrophilic drug ROS was observed (up to 50 %), while for the lipophilic drug EZE, EE up to 17 % was determined. Same trend for DC was observed, being up to 2.5% (out of theoretical value 4.4%) and up to 1.6% (out of theoretical value 7.9%) for ROS and EZE, respectively. With increase in the concentration of DSPE-PEG2000 while keeping other parameters constant, the EE of both ROS and EZE decreased, with a decrease being lower to insignificant for EZE in comparison to ROS (from 16% to 10% and from 50% to 11%, respectively). With increase in the concentration of soybean phosphatidylcholine up to 0.56 mg/mL while keeping the other parameters during synthesis constant, significant increase in the EE of ROS was observed (from 15 % to 50 %), while no significant changes in the EE of EZE were observed, being $15 \pm 2\%$. The same trend for DC was observed with increasing phospholipids concentrations. The increase in the concentrations of both DSPE-PEG2000 and soybean phosphatidylcholine led to formation of slightly more negative NPs, with no significant impact on the z-average.

However, significant increase in the PDI with increase of phospholipids concentrations was observed, although within a range pointing to a narrow size distribution. These effects lead to conclusion that the particles tend to form aggregates when the phospholipid to polymer ratio is increased and that the used amounts of phospholipids are already sufficient to stabilize the PLGA particles. When non-PEGylated DSPE was used instead of DSPE-PEG2000, no significant differences in ZP, PSD and PDI were observed (-1.31 mV vs. -1.14 mV, 124 nm vs. 138 nm and 0.285 vs. 0.341, respectively). However, significant impact on EE of both drugs was observed, with nearly 50% decrease in the EE of ROS (from 50% to 25%) and 60% decrease in the EE of EZE (from 16% to 10%), pointing to significant impact of drugs-PEG interactions on drugs loading.

Conclusion

In this paper, relatively simple and effective single step nanoprecipitation technique was used for synthesis of EZE/ROS loaded LPHNPs. All evaluated formulation variables [phospholipids (to polymer) ratio as well as the type of DSPE] had impact on physical characteristics of LPHNPs, significantly on the EE and DC, pointing to a need for further optimization of the formulation until obtaining the final desired characteristics.

References

- Nakjinova, N.V., Jovanovikj, N., Kavrakovski, Z., Geshkovski, N., Mladenovska, K., 2022. Influence of organic to aqueous phase solvent volume ratio on the physicochemical characteristics of rosuvastatin and ezetimibe loaded lipid-polymer hybrid nanoparticles prepared by nanoprecipitation method. *Maced. Pharm. Bull.* 68, 305 - 306. doi:10.33320/maced.pharm.bull.2022.68.03.147
- Tang, B., Qian, Y., Fang, G., 2020. Development of lipid-polymer hybrid nanoparticles for improving oral absorption of enoxaparin. *Pharmaceutics*, 12(7), 607. doi: 10.3390/pharmaceutics12070607.
- Chilbert, M.R., VanDuyn, D., Salah, S., Clark, C.M., Ma, Q., 2022. Combination Therapy of Ezetimibe and Rosuvastatin for Dyslipidemia: Current Insights. *Drug Design, Development and Therapy*, 2177-2186. doi:10.2147/DDDT.S332352