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Short communication

Bioavailability and metabolism of olive bioactives

Theodora Nikou[1], Maria-Eleni Sakavitsi[1], Christina Fytili[2], Nikolaos Tentolouris[2], Luc Pieters[3], Nina Hermans[3], Maria Halabalaki*[1]

[1]*Division of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, National and Kapodistrian University of Athens, Panepistimioupoli Zografou, 15771 Athens, Greece*
[2]*First Department of Propaedeutic and Internal Medicine, Medical School, National and Kapodistrian University of Athens, Laiko General Hospital, 17 Agiou Thoma Street, 11527 Athens, Greece*
[3]*Natural Products & Food Research and Analysis (NatuRA), Department of Pharmaceutical Sciences, University of Antwerp, Belgium*

Introduction

Olive products are chemically characterized by characteristic secoiridoids and phenylalcohols (biophenols), widely known for their significant health beneficial effects (Bulotta *et al.*, 2014). Their bioactivity is directly related to their metabolism and biotransformations in human gastrointestinal system, demonstrating their mechanism of action, efficacy and safety. Additionally, it is evident that gut microbiome is one of the major factors affecting the metabolic fate and final bioavailability of orally administered compounds, and therefore its investigation is in the center of scientific research.

So far limited and scattered data exist for the metabolism of olive biophenols and further exploration is required to associate their bioactivity with their metabolites and ultimately the true active entities. Commonly, LC-MS platforms are incorporated for the analysis of human biofluids, while chemometrics and metabolomics approaches have been proven as powerful tools suggesting a holistic mining and identification of biomarkers and/or biosynthetic pathways for the investigation of metabolic patterns (Piroddi *et al.*, 2017).

Aiming towards a holistic experimental workflow for the exploration of olive products metabolism, pure hydroxytyrosol, tyrosol, oleacein, oleocanthal and oleuropein were investigated *in vitro* using a continues GastroIntestinal Dialysis Model with colon phase (GIDM-colon). The model approximates the metabolism of orally administered compounds simulating gastric, small intestine and colon phase (Sakavitsi *et al.*, 2022).

In parallel, hydroxytyrosol was supplemented for a six-months period in the form of soft capsule (15 mg HT/day) in a randomized double-blind prospective human study and urine samples were collected in three-time points (Fytili *et al.*, 2022). *In vitro* and human samples were analysed using LC-HRMS & HRMS/MS and metabolomics analyses to unravel the metabolism of olive bioactives, map their metabolic pathways and discover their biotransformation reactions and metabolites in human organism.

Materials and methods

In vitro GIDM-colon metabolism

Hydroxytyrosol, tyrosol, oleacein, oleocanthal and oleuropein after isolation and purification were subjected to GIDM-colon model in the amount of 15 mg per compound. The model was consisted of chambers-cells mimicking the human gastrontesinal digestion and the respective conditions (gastric phase, small intestine and large intestine) in terms of the pH, temperature, oxygen availability, enzymes and human mictrobiota. In each chamber, solutions were incubated for the appropriate time range depending on the respective metabolic stage (1 h for gastric; 2 h for small intestine phase and 24 h for colonic phase) and samples were collected in different time points.

Human intervention study

Hydroxytyrosol was administered as a soft capsule to

*mariahal@pharm.uoa.gr

28 momen in the dose of 15 mg/day. Participants were randomized in two study groups (HT group and placebo) and urine samples were colected in three time points (T = 1 month, T = 3 months and T = 6 months). Samples were analyzed with LC-HRMS.

LC-HRMS based profiling and dereplication

In vitro and human urine samples were analyzed via UPLC-IT-Orbitrap MS. Acetonitrile and acidified water with formic acid were used as mobile phase and a C-18 column was incorpoarated for the chromatographic preparation. Sampes were analyzed in negative ionization and mass spectrometric parameters were optimized and applied as follows: sheath gas 40 ard; aux gas 10 arb; capillary temperature 350^{o}C; capillary voltage: -30 V, tube lens: -100 V; scan range 115-100 *m/z*.

Multivariate data analysis (MVA) and metabolomics

LC-HRMS data were recorded with Xcalibur 2.2 and then imported to MZmine software for data processing. All the appropriate modules were employed for the peak lists generation. A detailed dereplication methodology was applied for the identification of metabolites. The final lists were imported into SIMCA software for statistical analysis. Mainly principal component analysis (PCA) and orthogonal partial least squares (OPLS) methods were implemented for model generation a.

Results and discussion

Initially, MVA analysis of GIDM-colon model data revealed classification trends for each metabolism phase (gastric, intestine, colon) per tested compound revealing significant information about their absorption and metabolic biotranformation and therefore bioavailability. Dereplication and suspect analysis in all samples also enabled the identification and putative annotation of new metabolites of the respective precursor compounds. Chemical biotrasformations of each compound and gastrointestinal phase were correlated with the respective chemical structure of the parent compounds, revealing interesting data for secoiridoids and phenylalcohols metabolism. Finally, the comparative study across the tested compounds and based on their structural motifs allowed the generation of reasonable hypotheses regarding the chemical scaffolds ranking for optimum bioavailability. Regarding the human study similar workflow was followed for the visualization of data and annotation of metabolites. the respective peak lists were generated. MVA analyses revealed certain biomarker compounds found as statistically significant of hydroxytyrosol and placebo group, respectively. The identified compounds gave better insight into metabolic changes of urine metabolome and exposed the preferred metabolic pathways of compounds after hydroxytyrosol administration as well as their final bioavailability in human organism.

Conclusion

LC-HRMS & HRMS/MS metabolomic and dereplication approaches on *in vitro* and human data revealed key metabolites and biomarkers of hydroxytyrosol metabolism and its derivatives. Significant insight was given into the bioavailability of olive bioactives in human body focusing on metabolism while biomarker compounds associated with consumption of olive products facilitate the elucidation of their mechanism of action. This approach could serve as a model workflow for the study of food bioactive compounds underlining their role in diet as well as their beneficial effect on health. Finally, it could be used as an inspiration for drug development towards the discovery of new actives agents.

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