AKTION Czech Republic - Austria report

84p16 - Metabolism of compounds formed by intestinal microflora

The aim of the cooperation between Department of Pharmacology in Olomouc and Division of Clinical Pharmacy and Diagnostics in Vienna was to deepen the knowledge about metabolism of natural compounds (such as anthocyanins) in the intestinal cells and to find out whether metabolites formed by intestinal bacteria can easily pass through intestinal barrier or whether they are further metabolized in the cells. In Vienna, within the project, human epithelial colorectal adenocarcinoma cells (Caco-2 cells) were incubated with four compounds (4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 2,4,5-trihydroxybenzaldehyde, or 2,4,6-trihydroxybenzaldehyde) that was formed by intestinal bacteria from anthocyanins as we have already found out. The incubations were set to 37 °C for 6 or 20 hours. After incubations, both the solutions as well as Caco-2 cells were frozen at -80 °C. All frozen samples were transported on dry ice to Czech Republic, where solutions and cells were subjected to the analysis of parent compound as well as of metabolites formed during incubation. Results show that 4-hydroxybenzoic acid was glucuronated while 3,4-dihydroxybenzoic acid was methylated by Caco-2 cells. 2,4,6-trihydroxybenzaldehyde was converted to two glucuronated metabolites and 2,4,5-trihydroxybenzaldehyde was glucuronated as well as methylated. Moreover, 2,4,6-trihydroxybenzaldehyde and its glucuronides were also detected inside cell lines. Therefore, compounds created in the intestine by intestinal bacteria can pass into intestinal barrier and can be metabolized in the intestinal cells. Suggested mechanisms of antocyanidins metabolism by intestinal bacteria and mechanisms of metabolism of formed metabolites by Caco-2 cells are included in the manuscript (enclosed) that will be submitted to the journal. This project helped to obtain new knowledge in the area of metabolism of natural compounds in the intestine.

Scientific participants in Vienna (Julia Gausterer and Stefan Poschner) performed experiment with Caco-2 cells (provided by Division of Clinical Pharmacy and Diagnostics in Vienna) and tested compounds (provided by Department of Pharmacology in Olomouc). They prepared Caco-2 cell lines and solutions with compounds, performed the incubation of Caco-2 cells with solutions, and prepared samples for the transport to the Czech Republic. Laboratory materials and instruments (microscope, UV/Vis spectrophotometer) were provided by Division of Clinical Pharmacy and Diagnostics in Vienna, flasks for Caco-2 cell lines growth

and chemicals (medium, antibiotics) which were necessary to achieve suitable conditions for cells growth during experiment were bought for 34 557.68 CZK. Participant in Olomouc (Zuzana Rácová) transported these samples to the Czech Republic and prepared them for the LC/MS analyses. Solutions, microtubes, vials, and guard precolumns for analyses were provided by Department of Pharmacology in Olomouc, an analytical column for separation of compounds in samples and an oil for vacuum pump of MS instrument were purchased for 36 970.06 CZK. Zuzana Rácová also evaluated data obtained from LC/MS instrument.

The visit of Prof. Walter Jäger (partner of cooperation) at the Department of Pharmacology in Olomouc was 9. - 12. 7. 2019. The visit was important for discussion about results obtained within the project. The accommodation in Olomouc for Prof. Walter Jäger cost 1 060 CZK/night and daily allowance was 600 CZK/day. The possibility of the participation at the congress with these results was also discussed with colleagues at the Faculty of Science of Charles University (as organizers of the congress) in Prague. Fare for Prof. Pavel Anzenbacher (project submitter), Prof. Walter Jäger (partner of cooperation), and Zuzana Rácová to Prague and back cost 2161 CZK. Results obtained within this project were included into manuscript (attached to this report). The discussion about the finishing of the manuscript was held 17. 12. 2019 at the Division of Clinical Pharmacy and Diagnostics in Vienna. The fare to Vienna and back for Zuzana Rácová cost 860.73 CZK.

Total approved subsidy in CZK was 115 676 CZK, 8 601.73 CZK was used for the accommodation and daily allowance for partner of cooperation and for all fares by bus or by train. 71 527.74 CZK was used for purchase of laboratory material which was used for experiments. It is more than the amount planned by 4 527.74 CZK and it was needed to be spent to cover the necessary expenses of the experiment. This amount was covered from the funds originally assigned to cover the travel expenses. 10 % of the amount (used to fund the project) was used for overhead costs. In total, 88 133.14 CZK was used for implementation of this project and remaining 27 542.86 CZK was not used. However, we have received a subsidy of 151 676 CZK and it is more than we planned for this project. For this reason, the undrawn amount is 63 542.86 CZK.

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