ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Pharmacology and Toxicology

Candidate: MSc. Lucie Zemčíková

Supervisor: Assoc. Prof. PharmDr. František Trejtnar, CSc.

Title of doctoral thesis: The effect of natural compounds on transport by OATP drug transporters

OATP membrane transporters belong to carrier proteins responsible for transporting certain drugs (e.g. hypolipidemics from the group of statins) and other xenobiotics across the biological membranes and tissue barriers within the body. These transport proteins play an important role in pharmacokinetic processes such as absorption, distribution and elimination. The potential modulation of their transport function by natural compounds commonly present in plant food or food supplements may result in the changes of the concentration of their substrate (drug) in the cells and body fluids, that may affect the effect and toxicity of these drugs.

The aim of this study was to obtain data on the interactions of selected natural compounds with human transporters OATP2B1 and OATP1A2 and their ability to affect drug transport mediated by these transporters. These two OATP transporters are involved in the drug uptake especially in organ barriers important for pharmacokinetics. For the study natural compounds from the group of flavonoids and isoflavones were selected. The common characteristics of studied flavonoids (fisetin, galangin, chrysin, myricetin, pinobanksin and pinocembrin) is their presence in the honey; studied isoflavones (daidzin, daidzein, genistin, genistein, glycitin, glycitein, biochanin A and formononetin) are mainly present in soya and soy products. The interaction of the most important isoflavone metabolites formed in gastrointestinal tract (*S*-equol, *O*-desmethylangolensin) with these transporters was also studied. The widely used hypolipidemic drug rosuvastatin served as model drug substrate of studied OATPs.

Selected cell lines transiently transfected with studied OATP transporters served as experimental models. The overexpression of studied transporters was verified. These cell models were used for the inhibitory studies to examine the effect of selected flavonoids and isoflavones towards the transport function of the studied transporters. The standard inhibitory parameters IC_{50} and K_i were used for quantitative characterization. Flavonoid quercetin served as a comparator, because its inhibitory activity towards studied OATPs has been already described. We also analyze the mechanism of inhibitory interaction of studied transporters by selected natural compounds. The potential to influence drug transport was studied by observing of the effect of selected flavonoids on uptake of radiolabeled drug rosuvastatin. The comparison of flavonoid cytotoxicity in cells overexpressing studied transporters and mock-cells was performed as indirect method of determination whether the flavonoids are also potential substrates of these transporters.

The results of *in vitro* inhibitory studies have shown that chrysin, galangin and pinocembrin are able to inhibit OATP2B1 and OATP1A2, respectively, at lower or comparable concentrations as quercetin. Galangin, chrysin and pinocembrin inhibited the rosuvastatin uptake mediated by OATP2B1 with the range of IC_{50} 1–10 μM. The inhibition of rosuvastatin uptake mediated by OATP1A2 by these flavonoids was lower. In the case of soy isoflavones, aglycones and the main biologically active metabolite *S*-equol significantly inhibited the OATP2B1-mediated transport with K_i values 1–20 μM. In contrast, glucoside forms did not exhibit statistically significant effect on OATP1A2-mediated uptake of standard substrate. The kinetic analysis not indicate the uniform type of inhibition of OATP by studied natural compounds, although predominant mechanism of inhibition seemed to be competitive especially in isoflavones. The transport of studied flavonoids by OATPs into the cells was not clearly proved by cytotoxicity assay.

The obtained data has shown the ability of range of the studied natural compounds to inhibit the transport mediated by OATP2B1 and/or OATP1A2 transporter *in vitro*. These findings may suggest the potential to affect transport and modify pharmacokinetics of drugs transported by OATP across the biological membranes even *in vivo*.