

ABSTRACT

Charles University in Prague, Faculty of Pharmacy in Hradec Králové

Department of Pharmacology and Toxicology

Candidate **Mgr. Jana Mandíková**

Supervisor **doc. PharmDr. František Trejtnar, CSc.**

Title of Doctoral Thesis **Interactions of antimicrobial agents with drug renal transport systems in vitro**

A number of important and frequently used antimicrobial agents are excreted from the body through the kidneys. During this excretory process drugs may interact with different membrane transport systems (transporters) which are expressed in the renal tissue. These interactions are often the determining factors for both renal excretion, and the toxic effects of these drugs in the kidney. There are highly expressed influx transporter such as organic anion transporters (OATs), organic cation transporters (OCTs) and the concentrative nucleoside transporters (CNTs) and efflux transporter BCRP (breast cancer resistance protein) and P glycoprotein (P-gp) in the kidney. Despite the importance of renal drug transporters for elimination and nephrotoxic effects of drugs and intensive research in this field, interactions of a number of important antimicrobial drugs with transporters in the kidney are not known or sufficiently described. The aim of this study was to investigate the interaction of selected antivirals and antibiotics with important human renal drug transporters in vitro, to assess the potential of these interactions for drug drug interactions with selected drugs and determine the importance of the found transport mechanisms for the toxicity of the studied compounds. An important part of the work was the implementation of the optimized methods using cell models with a transient increase in the expression of the studied transporters. These methods were used in inhibition and transport experiments, which served to determine the quantitative parameters of the interaction of the studied compounds with OAT1, OCT2, CNT2 and CNT3. Interaction of BCRP and P-gp with studied compounds were investigated using cell lines stably expressing high levels of these transporters. The research in this work was centered on the developmental antiviral agents from the group of acyclic nucleoside phosphonates (ANPs), the newer antiviral agent entecavir for the treatment of viral hepatitis and important antifungal agent amphotericin B in the complex with deoxycholate (AmB/DOC). Antivirals adefovir, tenofovir and cidofovir, which may be clinically administered simultaneously with the studied drugs, were used to compare the intensity of the interaction of the studied compounds with selected transporters and were studied in terms of potential drug interactions with the tested substances. In vitro studies demonstrated that entecavir (ETV) is an inhibitor and substrate of hOAT1, hCNT2 and hCNT3. ETV inhibited the cellular uptake of adefovir, tenofovir and cidofovir via hOAT1. However, the effective inhibition of transport was observed at concentrations of ETV highly exceeding therapeutic levels. In comparison with adefovir, tenofovir and cidofovir, ETV was less toxic to cells transfected with hOAT1. ETV showed a mild inhibitory effect on BCRP/ABCG2 and P gp/ABCB1. Most of the tested ANPs interacted with hOAT1. The affinity of the substance GS 9191 for hOAT1 was relatively high and comparable to the affinity of the known substrates of hOAT1 adefovir and tenofovir. The rate of interaction with hOAT1 correlated with the lipophilicity of these compounds. While GS-9191 showed strong inhibitory effects on the function of P gp and BCRP, PMEOPy inhibited only P gp. Most of the tested ANPs caused a significant decrease in viability of the cells transfected with hOAT1. Cytotoxic effect of ANPs in vitro, with the exception of the relatively lipophilic GS 9191, corresponded to their ability to interact with hOAT1. Increased cytotoxicity of ANPs with the affinity for hOAT1 is an indirect evidence that these substances are not only inhibitors but also substrates of this transporter. Performed accumulation studies confirmed active transport of AmB into cells. AmB/DOC inhibited both hOAT1, and hOCT2. We identified DOC as the component responsible for the interaction with OAT1. AmB did not interact with hOAT1, but significantly inhibited hOCT2. We did not find the cellular uptake of AmB via hOAT1 and hOCT2. AmB/DOC in supratherapeutic concentrations interacted with adefovir, tenofovir and

cidofovir in the cells transfected with hOAT1.

In conclusion, ETV in comparison with nephrotoxic antivirals showed relatively weak interactions with the studied renal transporters, which are not likely to be relevant to transporter mediated cytotoxicity nor are the basis for drug-drug interactions. Among the tested ANPs, we found the differences in interactions with hOAT1. This transporter could be relevant for the renal excretion of ANPs in vivo. The performed studies have demonstrated the first interaction of AmB with membrane transport system, which is probably not included in secretion of AmB in renal tubules, but its importance for drug interactions and drug toxicity should be verified in the future.