ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové

Training Workplace	Department of Pharmacology and Toxicology
Doctoral Degree Program	Pharmacology and Toxicology
Candidate	Mgr. Martin Huličiak
Supervisor	Assoc. Prof. PharmDr. Lukáš Červený, PhD.
Advisor	PharmDr. Ivan Vokřál, PhD.
Title of Doctoral Thesis	Study of intestinal transporters and their role in drug absorption

Oral drug administration is a widely accepted method due to its convenience and cost-effectiveness, with the small intestine playing a crucial role in drug absorption, thus affecting bioavailability and serving as a potential site for drug-drug interactions (DDIs). Efflux drug transporters such as P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2), along with metabolizing enzymes such as cytochrome P450 3A4 (CYP3A4) at the intestinal barrier, significantly contribute to DDIs. Risk factors for DDIs include comorbidities, advanced age, and polypharmacotherapy. People living with HIV requiring antiretroviral therapy (ART) are particularly susceptible to DDIs, especially when co-infected with HCV. Antivirals, commonly used in ART and in the treatment of HCV infection, can interact with efflux transporters and CYP enzymes, affecting drug efficacy and safety. Therefore, this study mainly focuses on the DDIs of antivirals on the intestinal efflux transporters.

Precision-cut intestinal slices (PCIS) offer a valuable *ex vivo* model for studying drug transport and metabolism in the intestine, closely mimicking *in vivo* conditions. We employ this method along with FDA-recommended *in vitro* bidirectional transport studies across Caco-2 monolayers. Taking into account the distinct binding sites within the ABCB1 binding pocket, we used multiple probes to assess the inhibitory potency of antivirals. Several antivirals, mainly protease inhibitors (PIs), inhibited ABCB1-mediated transport in both *in vitro* and *ex vivo* models.

Furthermore, the study explored the effects of sofosbuvir on the absorption of tenofovir and its prodrugs, tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide fumarate (TAF). TDF and TAF are likely to produce similar tenofovir concentrations in the portal vein, with ABCB1 influencing their permeation extent. In addition, sofosbuvir may enhance TDF stability in the intestinal environment.

The PCIS model also proved to be valuable for studying drug induction toward intestinal enzymes and transporters, aiding in understanding potential DDIs.

In vitro and *ex vivo* studies revealed the inhibitory effects of antivirals on intestinal ABCB1-mediated transport, highlighting the need to evaluate drug interactions. The use of multiple probes is crucial to a complete assessment of the drug inhibitory potential. Our findings also demonstrate the utility of the PCIS technique in inhibition and induction studies focused on ABCB1. This method may also be valuable in quantifying the involvement of intestinal ABCB1 and DDIs in presystemic drug elimination.