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## Drug transport and transport-metabolism interplay in the human and rat intestine

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# Chapter 1

## Introduction

## General introduction

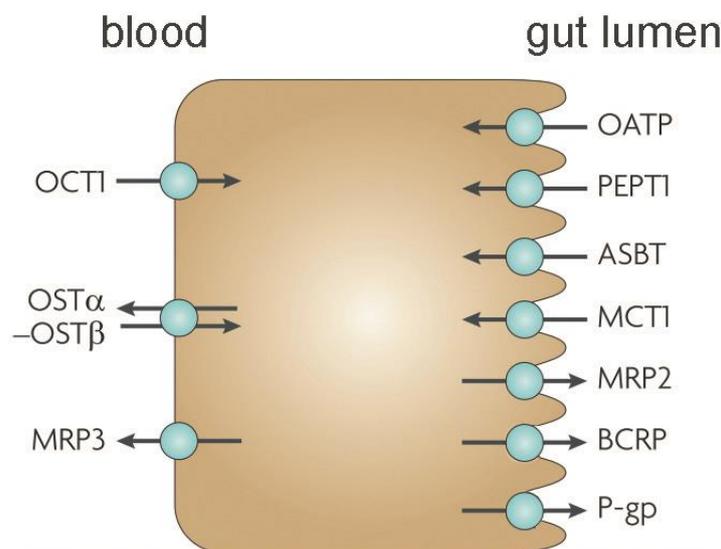
The intestine is the main location for drug absorption after oral administration, whereas its role in drug metabolism, distribution, excretion and toxicity (ADME-tox) has been intensively studied and is well acknowledged in the last decades [1-3]. However, in contrast to the liver, the intestine is a very heterogeneous organ and its involvement into the ADME-tox process is considerably different in the different intestinal regions, e.g. duodenum, jejunum, ileum and colon. These differences are not merely on the tissue/organ level, e.g. different structure, blood flow, lumen pH etc. [4], but also on the cell and molecular level, e.g. different expression profiles of drug transporters (DTs) and metabolizing enzymes (DMEs) [5, 6]. Thus, the influence of DTs on the ADME-tox in the various regions of the intestine must be taken into account in drug development [7, 8].

## Intestinal transporters

Although the intestinal absorption of many drugs occurs for an important part via passive diffusion, intestinal transporters also play important roles for a large group of compounds and is even the dominant route for certain classes of drugs [9-11]. The most important DTs that are expressed and located in the intestine are shown in **Fig. 1**.

Influx transporters, located on the apical membrane of intestinal epithelial cells, facilitate intestinal absorption [5]. Many of them are members of the solute carrier (SLC) super-family. For instance, peptide transporter 1 (PEPT1; SLC15A1) is the primary uptake transporter for di-/tri- peptides; organic anion transporting polypeptides (OATPs; SLCOs) mediate the transport of a diverse range of amphiphilic organic compounds sodium-independently; and the organic cation transporter 1 (OCT1; SLC22A1), transports relatively hydrophilic, low molecular mass organic cations. In addition, the apical sodium-dependent bile acid transporter (ASBT; SLC10A2) plays an indispensable role in the intestinal absorption of bile acids, as part of the enterohepatic recirculation [12]. It reabsorbs approximately 95 % of the luminal bile acids in the ileum [13-15]. Both conjugated and unconjugated bile acids can be taken up by ASBT but they differ in affinity for ASBT. Furthermore, malfunction of ASBT can lead to inflammatory bowel disease, constipation and Alagille syndrome, therefore ASBT is also a determinant in these bile acid-related diseases [16, 17]. In the human small intestine ASBT is solely localized on the apical membrane of the ileum whereas rat ASBT is also expressed in the caecum [18].

# Intestinal epithelia



**Fig. 1** The transporters expressed and located on the apical (lumen side) and basolateral (blood side) membrane of intestinal epithelia. Uptake transporters on the apical membrane are the organic anion transporting polypeptide (OATP) family, the peptide transporter 1 (PEPT1; SLC15A1), the ileal apical sodium/bile acid co-transporter (ASBT; SLC10A2), and monocarboxylic acid transporter 1 (MCT1; SLC16A1). The apical ATP-dependent efflux pumps include multidrug resistance protein 2 (MRP2; ABCC2), breast cancer resistance protein (BCRP; ABCG2) and P-glycoprotein (P-gp; MDR1, ABCB1). The basolateral membrane of intestinal epithelia contains organic cation transporter 1 (OCT1; SLC22A1); the heteromeric organic solute transporter (OST $\alpha$ -OST $\beta$ ), and MRP3 (ABCC3). Reproduced with permission from [9].

Efflux transporters, located at both the basolateral and apical membrane, facilitate efflux of compounds into the blood stream and the lumen respectively. The organic solute transporter (OST $\alpha$ -OST $\beta$ ) mediates efflux of bile acids into the bloodstream. P-gp, BCRP, and the members of the MRP family, belong to the ATP-binding cassette (ABC) family and can excrete their substrates out of the cells into the intestinal lumen against the concentration gradient by using the energy from ATP hydrolysis, thereby lowering the intracellular concentration and, when located at the apical membrane, decreasing intestinal absorption [19-21]. P-gp plays a key role due to its broad substrate specificity and high expression level. P-gp activity can significantly influence the intracellular concentration of both the parent drug and its metabolite(s) in the enterocyte, thereby also determining the systemic exposure to these compounds. This is crucial for drugs with a narrow therapeutic window, such as digoxin, since relatively

small fluctuations of its blood plasma concentration can lead to either therapeutic failure or toxic effects [22]. Furthermore, the level of expression and functionality of P-gp can be modulated by induction and inhibition. As a result, it is of importance to evaluate for every new chemical entity (NCE) whether it is a substrate and/or inhibitor of P-gp and if so, its affinity and/or inhibitory potency [9]. In addition, it is also important to take the regional differences into the consideration for this evaluation, as the P-gp expression is reported to exhibit a gradient ileum > jejunum > duodenum  $\geq$  colon [23, 24]. Since P-gp is a target for a wide spectrum of inhibitors and has broad and overlapping substrate specificity with some of the DMEs, clinically important drug-drug interactions (DDIs) related to P-gp activity occur frequently [25, 26].

### **Intestinal metabolizing enzymes**

In the last two decades, the metabolic capacity of the intestine and its contribution to first pass metabolism has been increasingly recognized. Studies *in vivo* demonstrate that this significant intestinal metabolism has implications for the bioavailability of drugs [27]. In human intestine, the clinically important DMEs include cytochrome P450 (CYP) oxidases and the flavin-containing monooxygenase (FMO), UDP-glucuronosyltransferases (UGTs) [28], glutathione S-transferases (GSTs) [29], and sulfotransferases (SULTs) [30]. This system of enzymes acts in two stages to firstly oxidize the xenobiotic (phase I) and then conjugate water-soluble groups onto the molecule (phase II). Thus, the modified water-soluble xenobiotic can be pumped out of cells (considered as phase III nowadays) and can then be excreted from the body via the urine or bile.

CYP-enzymes, the most important DME family, play a central role in the hydroxylation, dealkylation and oxidative metabolism (phase I) of clinically used drugs and other xenobiotics [31]. Among the CYP superfamily, the CYP3A subfamily accounts for about 30 % of total CYP-enzymes and involves in the metabolism of approximately 50 % of all therapeutic drugs on the current market [31]. Also CYP3A has a broad substrate specificity, is a target for many inhibitors and its expression can be induced by many compounds. CYP3A shows the highest expression in the proximal intestine, which decreases towards the ileum with the lowest expression in colon [32]. Data on metabolic activity in the different regions are scarce as it is difficult to obtain these data in man *in vivo*.

### Transport-metabolism interplay

In the intestine, DTs, especially efflux transporters, and DMEs share a physiological function, which is to decrease the absorption of xenobiotics including drugs. Furthermore, they can work coordinately, which is currently known as the transport-metabolism interplay, to complete this barrier function in an efficient way, either based on the overlapping substrate specificity (parallel interplay) or based on the fact that products of DMEs are substrates of DTs (concatenated interplay) [33].

Due to the fact that both P-gp and CYP3A have a significant influence on the ADME-tox process, the P-gp/CYP3A interplay raised a lot of interest and was widely studied. Such interplay has been postulated based on experiments in Caco-2 cells for shared P-gp and CYP3A substrates [34], and it is believed that their coordinated activities can further reduce the intracellular concentrations of xenobiotics and therefore lower the intestinal absorption of drugs [35]. However, the consequence of the P-gp/CYP3A interplay *in vivo* is still under debate till now as controversial results have been found. Watkins et al. hypothesized that P-gp may prolong the residence time of its substrates in the intestine, therefore increasing the possibility for intestinal metabolism [36]. Supporting the same conclusion, other explanations also exist. For instance, P-gp is believed to excrete also the CYP3A metabolites, as they are often also P-gp substrates, from the enterocytes, thereby reducing the chance of product inhibition. Consequently, P-gp inhibition would decrease the total intestinal metabolism. On the contrary, Pang et al. stated that P-gp efflux limits metabolism due to the competition between P-gp and CYP3A within the cell and that their interplay is independent of the mean residence time of drug in the system [37]. Thus, they conclude that P-gp inhibition will increase metabolism, since the intracellular substrate available to CYP3A will be increased [38]. Similarly, when P-gp limits the absorption in the proximal small intestine, the absorption is shifted to more distal, less catalytically efficient segments that contain lower amounts of CYP3A [36, 39], thus P-gp inhibition will increase metabolism, but only under conditions where the CYP3A is not saturated. However, the effect of the interplay in the different regions of the intestine has not been studied in detail and evidence of P-gp/CYP3A4 interplay in the human intestine *ex vivo* is also lacking.

Transport-metabolism interplay was also suggested for MRP2/UGT, MRP2/GST, and

BCRP/SULT but has been much less investigated. This interplay is not based on a common substrate but in these cases the metabolite is the substrate for the transporter. Phase II DMEs, such as UGT, GST, and SULT, produce drug-conjugates which have much higher hydrophilicity and show high affinity for MRP2 and BCRP. Thus, the intracellular concentration of the absorbed drug quickly decreases by the metabolism and excretion of the metabolite prevents accumulation and associated product inhibition of the enzyme. Therefore, inhibition of MRP or BCRP may increase the concentration of the drug-conjugates in the cell, thereby exerting feedback inhibition of the metabolism [33].

### **Models for the intestinal transport and/or metabolism**

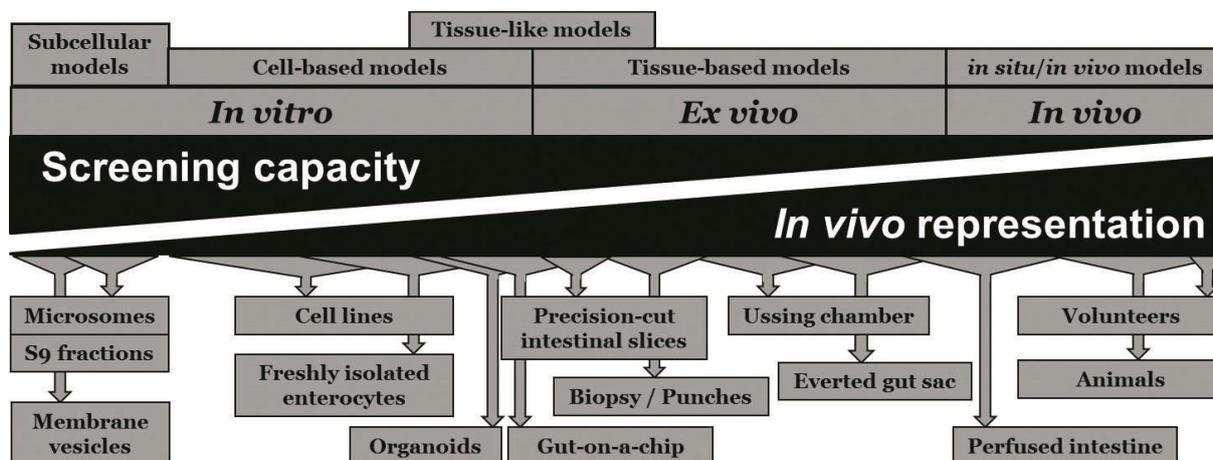
Many methods, as shown in **Fig. 2**, have been developed and applied to characterize and predict the ADME-tox properties in the intestine of NCEs [9, 19, 40-43]. *In vitro* cell cultures and *in vivo* animal models are the conventional methods which are widely used in both academia and industry, and they are well characterized. However, *in vitro* cell cultures do not reflect the tissue multi-cellularity, 3D structure, physiological expression levels of DTs and DMEs, and some cell types lose their polarization during culture. On the other hand, the *in vivo* models retain the proper physiological conditions, their screening capacity is too low and the cost, both in animal lives and in money, is much higher. Moreover applications of these *in vivo* models in human are extremely difficult due to technical and ethical constraints and exceptionally high costs. To fill this gap, alternative methods using intact tissue, such as everted sac, perfused intestinal loops, Ussing chamber, intestinal punches and, more recently, precision-cut intestinal slices (PCIS) have been developed and are increasingly used, to provide additional information on the intestinal handling of NCEs during pre-clinical investigation.

### **Precision-cut intestinal slices**

Precision-cut intestinal slices (PCIS) have been well-established for drug metabolism, induction and toxicity in the intestine *ex vivo* [44-48]. However at the start of this study no paper had been published on drug transport or transport-metabolism interplay studies in PCIS yet.

The applications of precision-cut intestinal slices in drug metabolism, transport and toxicity, including the studies described in the thesis, are introduced and extensively discussed in

## Chapter 7.



**Fig. 2** Schematic classification of the current models to study the ADME-tox properties drugs and xenobiotics in the intestine. Reproduced with permission from [49]

### The scope of the thesis

The aim of the research described in this thesis is to investigate the transport and transport-metabolism interplay in human and rat intestine *ex vivo* using precision-cut intestinal slices.

The following research questions were studied:

- Can PCIS from the rat and human intestine serve as a model to study uptake and efflux transporters?
- Are the regional differences in transporter expression as reported in the literature reflected in functional differences?
- What is the effect of P-gp inhibitors on the P-gp/CYP3A interplay?
- What are the species differences between human and rat intestine with respect to transport function and P-gp/CYP3A interplay?

In **Chapter 1**, the background of the research is introduced; including the transport, metabolism and their interplay in the intestine as well as precision-cut intestinal slices PCIS as *ex vivo* model.

In **Chapter 2 and 4** PCIS are validated as an *ex vivo* model to investigate P-gp activity and inhibition in rat and human intestine, respectively. In addition, the potencies of several P-gp inhibitors in the different regions of the intestine are presented and a broader range of

inhibitors was investigated in PCIS of rat ileum and human jejunum. The species differences of P-gp activity and inhibition are discussed in **Chapter 4**.

Based on the work of **Chapter 2**, the consequences of DDIs influencing the P-gp/Cyp3a interplay are further explored in the different regions of the rat intestine in PCIS in **Chapter 3**. Similarly, the P-gp/CYP3A4 interplay and its related DDIs with P-gp inhibitors in human PCIS are presented in **Chapter 5**. In addition, the correlation between the magnitudes of the response and the absolute P-gp abundance is discussed.

In **Chapter 6**, the use of PCIS to study the function of uptake transporters is validated. The function of the apical sodium-dependent bile acid transporters (ASBT), an important influx transporter in the intestine, is presented by analyzing the uptake of three different bile acids in rat and human PCIS.

In **Chapter 7**, the PCIS technology is reviewed and discussed as an alternative method for drug transport, metabolism and toxicology research.

In **Chapter 8**, the findings in **Chapter 2 - 6** are summarized and discussed, followed by suggestions for future research and future perspectives

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