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

Li, Ming

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Chapter 6

Human and Rat Precision-Cut Intestinal Slices
as Ex Vivo Models to Study Bile Acid Uptake by
the Apical Sodium-Dependent Bile Acid Transporter

Ming Li\textsuperscript{a}, Ivan Vokral\textsuperscript{b}, Bernard Evers\textsuperscript{a}, Inge A.M. de Graaf\textsuperscript{a}, Marina H. de Jager\textsuperscript{a}, Geny M. Groothuis\textsuperscript{a}

Ming Li, Ivan Vokral and Bernard Evers contributed equally to this work

\textit{(Manuscript in preparation)}

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Inge A. M. de Graaf
ABSTRACT

The apical sodium-dependent bile acid transporter (ASBT) is the primary transporter for the uptake of bile acids in the small intestine. It is localized on the apical membrane of the ileal enterocytes and is known for its high capacity and affinity for taurocholic acid (TCA) in vitro. However, less is known about its activity towards TCA and other bile acids in vivo due to the lack of a suitable model. The aim of this study was to validate the precision-cut intestinal slices (PCIS) using rat and human intestine as ex vivo models to study the activity of ASBT transport, and subsequently to study regional and interspecies differences in ASBT function. PCIS maintain the natural cell polarization and communication, expression of transporters and metabolizing enzymes, therefore this model is expected to be more relevant to the in vivo situation than in vitro cell culture models. PCIS of human and rat ileum were prepared and incubated with 0.04 - 2.00 mM TCA, deoxycholic acid (DCA) and cholic acid (CA), respectively at 4 and 37 °C. In this study the respective contribution of active uptake by ASBT appeared to be higher for TCA whereas the passive diffusion was higher for DCA and CA. Furthermore, the rank order of calculated apparent $K_{m}$ and $V_{max}$ is in line with literature reports. These results suggest that PCIS are a suitable model to study the activity of ASBT. In both species, the ASBT-mediated active uptake of bile acids was observed only in the ileum whereas only passive diffusion was observed in the jejunum and colon. In addition, ASBT activity was higher in the human ileum compared to the rat ileum. In the future rat and human PCIS can be used to study the uptake of new ASBT substrates and as a screening method for potential ASBT inhibitors.

Keywords: human and rat precision-cut intestinal slices; ex vivo; apical sodium-dependent bile acid transporter; uptake kinetics
INTRODUCTION

Major routes for the uptake and excretion of endogenous and exogenous compounds are passive diffusion and active transport by influx and efflux transporters. In the intestine multiple transporters are expressed on both the apical and basolateral membrane of the enterocytes. The apical uptake transporters in the small intestine play an important role in facilitating the influx of various nutrients, bile acids, drugs and their metabolites that are substrates of these uptake transporters [1]. The apical sodium-dependent bile acid transporter (ASBT), a member of the solute carrier (SLC) super-family encoded by the SLC10A2 gene, is such an uptake transporter [2]. In human small intestine ASBT is solely localized on the apical membrane of the ileum whereas rat ASBT, also known as Asbt, is primarily expressed on the apical membrane of rat ileum but also expressed in rat caecum [3].

ASBT is a major determinant in maintaining bile acid homeostasis, since it reabsorbs approximately 95% of the luminal bile acids in the ileum [4-6]. It is also well known for its high capacity transport of conjugated bile acids from the intestinal lumen into ileal epithelium [7-11]. Both conjugated and unconjugated bile acids are transported by ASBT but they differ in affinity for this transporter. Conjugated bile acids, which are the most abundant and more hydrophilic [12, 13], are more efficiently transported by ASBT [14], compared to the more hydrophobic unconjugated bile acids. Furthermore, dihydroxy bile acids (chenodeoxycholic acid (CDCA) and deoxycholic acid (DCA)) generally have a higher affinity for ASBT than the trihydroxy bile acids (cholic acid (CA) and taurocholic acid (TCA)) [11, 15, 16]. 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 [17, 18].

Therefore, ASBT has been an attractive target for drug development since it was discovered and identified, and it was hypothesized that the regulation of ASBT activity could serve as a treatment for several intestinal diseases, type 2 diabetes and hypercholesterolemia [19, 20]. For example, elobixibat (A3309), a selective and partial ASBT inhibitor, has been developed against chronic constipation and constipation-predominant irritable bowel syndrome, and GSK2330672, a highly potent ASBT inhibitor, for the treatment of type 2 diabetes, due to the influence of bile acids on glucose homeostasis, influencing glucagon-like peptide secretion. Both compounds are now in clinical trials [20, 21]. Moreover, ASBT has been shown to be a promising
target for drug-bile acid conjugates as prodrugs because of its high capacity and affinity for bile acids [22]. Bile acids can thus serve as “Trojan Horses” by coupling a drug molecule to a bile acid [23]. Thereby the oral availability of such a drug will be enhanced.

Multiple tools have been developed to study bile acid uptake by ASBT. ASBT function has been studied with ASBT-expressing (CHO, COS, HEK and MDCK) cells, oocytes, and membrane vesicles derived from those cells [16, 24, 25]. However, these in vitro models differ in many aspects from the conditions in vivo. Others have reported the use of wild type or genetically modified animals and in situ perfusion models [25, 26]. However, animal experiments are costly and a large number of animals are required for statistical evaluation as it allows only one experiment per animal. Precision-cut intestinal slices (PCIS) are a well-established ex vivo model to investigate drug metabolism, toxicity, and recently efflux transport in the intestine, allowing hundreds of tests per animal, thereby contributing to the reduction in animal use. Moreover this technique can be applied to human intestine with a tiny piece of tissue, which is obtained as medical waste after surgical resections. The use of PCIS has some other advantages. First of all, each slice contains all types of cells in the tissue in their natural environment, and consequently the natural cell polarization and communication and expressions of transporters and metabolizing enzymes are maintained [27-29]. Because the intestinal drug metabolism functions are represented well in PCIS [28, 30], they are expected to be a suitable ex vivo model to study uptake in the intestine.

For this reason we hypothesize that PCIS could provide new insight in ASBT-mediated transport in the intestine, as this model is expected to be more relevant to the in vivo situation than results from in vitro cell models. Therefore, the aims of this study were: (1) to validate rat and human PCIS as models to study the activity of ASBT; (2) to map regional differences in bile acid uptake; (3) to investigate species differences in bile acid uptake.

Three different bile acids, i.e. CA, TCA, DCA, were used in this study because of their differences in physicochemical properties regarding the number of hydroxyl groups. CA and TCA are trihydroxy bile acids while DCA is a dihydroxy bile acid. Furthermore, they represent three categories of bile acids: a primary bile acid (CA), a secondary bile acid (DCA), and a conjugated bile acid (TCA: conjugate of CA and taurine) [31, 32].

MATERIALS AND METHODS
Chemicals
Sodium taurocholate hydrate, sodium deoxycholate, agarose (low gelling temperature, type VII-A), Trizma® hydrochloride (TRIS) and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich Co. LLC (USA). William’s Medium E (WME) with glutamax-I, fungizone® antimycotic (amphotericin B) and gentamicin were purchased from Gibco® (UK). D(+) -glucose monohydrate, sodium hydroxide, EDTA together with sodium chloride and cholic acid sodium salt were derived from Merck Millipore (USA). HEPES was purchased from MP Biomedicals (Germany), methanol and ethanol absolute were from VWR Chemicals (USA). Both hematoxylin and eosin were purchased from Klinipath (the Netherlands). Bile acid stock solutions were prepared in DMSO.

Animals
All experiments were approved by the Animal Ethical Committee of the University of Groningen. Male Wistar (HsdCpb: WU) rats with a body weight ranging between 300 - 350 g were obtained from Harlan (Horst, the Netherlands). The rats were housed in a temperature-and humidity controlled room and fed ad libitum for at least 7 days before the experiment.

Human intestinal tissue
The use of human tissue was approved by the Medical Ethical Committee of the University Medical Centre Groningen. The pieces of jejunum were derived from patients undergoing a pylorus-preserving pancreaticoduodenectomy (PPPD). Ileum and colon were obtained from patients undergoing a hemicolec tomy. In some cases, during this procedure, a piece of colon and a piece of ileum were collected from the same patients. The characteristics of the donors are listed in Table 1. Immediately after resection the human tissue was stored in carbogenated ice-cold Krebs-Henseleit buffer (supplemented with 10 mM HEPES and 25 mM D-glucose, pH 7.4) and transported to the laboratory within 20 minutes.

Preparation and incubation of rat and human PCIS
Precision-cut intestinal slices from human and rat were prepared as previously described [27, 33]. Rats were anaesthetized with isoflurane/O₂ and exsanguinated via the aorta. The whole intestine was removed from the rat and directly stored at ice-cold carbogenated Krebs-Henseleit buffer. Rat intestine was divided into the duodenum, jejunum, ileum and colon. The first 2 to 12 cm distal from the pylorus was used as duodenum and the part
between 20 and 40 cm as jejunum. The last 10 cm proximal from the ileocecal junction was used as ileum and the part after ileocecal junction as colon. In case of human intestine, the muscle layer was removed and the mucosa layer was cut into segments of 10 × 20 mm before embedding. Both human and rat intestine were embedded using a tissue embedding unit (Alabama R&D, USA) into agarose solution (3 % agarose in 0.9 % NaCl) at 37 °C, which solidified on ice. The resulting cores were sliced using the Krumdieck tissue slicer (Alabama R&D, USA) into precision-cut slices with a wet weight of 3 - 4 mg representing a thickness of 300 - 400 µM. After removal of the agarose with a spatula, the individual slices were pre-incubated for 30 minutes in a 12-well culture plate (Greiner Bio-One GmbH, Austria) containing 1.3 ml WME supplemented with fungizone (2.5 µg/ml), gentamicin (50 µg/ml) and D(+) - glucose (25 mM). Depending on the aim of the experiment, the 12-well culture plates with slices were placed in plastic boxes in a cabinet with carbogen (95 % O₂ / 5 % CO₂), either at 37 °C or at 4 °C (on ice). A reciprocal shaker was used at approximately 90 times per minute (Reciprocating Shaker 3018, Gesellschaft für Labortechnik GmbH, Germany).

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Gender</th>
<th>Age</th>
<th>Region</th>
<th>Surgical Procedure</th>
</tr>
</thead>
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<td>1</td>
<td>F</td>
<td>66</td>
<td>ileum &amp; colon</td>
<td>hemicolecotomy</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>64</td>
<td>jejunum</td>
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<td>4</td>
<td>F</td>
<td>50</td>
<td>jejunum</td>
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<td>51</td>
<td>ileum</td>
<td>hemicolecotomy</td>
</tr>
</tbody>
</table>

**Intracellular ATP**

The viability of the slices was evaluated by measuring the intracellular ATP content [29]. After slicing, three slices were directly snap-frozen in 1 ml sonification solution (ethanol 70% v/v containing 2 mM EDTA, pH 10.9) in liquid N2 as control (fresh slices). Furthermore, the ATP content of control slices (t=0) was determined after 30 min of pre-incubation without bile
To test the toxicity of the bile acids, the ATP content was measured after incubation with or without bile acids at concentrations up to 2.00 mM, for 60 minutes for TCA and CA or for 10 minutes for DCA, as all concentrations DCA were toxic for slices after 60 minutes of incubation. The samples were stored at -80 °C until the ATP determination. The intracellular ATP content of the samples was assessed using the ATP Bioluminescence Assay Kit CLS II (Roche Applied Sciences, Germany) and a Spectramax micro-plate reader (Molecular Devices, USA).

### Evaluation of slice histomorphology

Rat PCIS were collected after the exposure to bile acids as described above and fixed in 4 % (v/v) formalin for 24 h at 4 °C. After 24 h the slices were transferred into 70 % ethanol (v/v) and stored another 24 h at 4 °C, followed by dehydration by submersion in solutions with increasing ethanol percentage up to 100%. After dehydration, PCIS were embedded in paraffin and 4 µm sections were cut. Hematoxylin and eosin staining was performed as described previously [34].

### Kinetics of bile acid uptake

The kinetics of bile acid uptake were assessed at 37 and 4°C [24]. Active uptake was estimated by subtracting the passive uptake at 4 °C from the total uptake at 37 °C [35]. After 30 min of pre-incubation, 40 µM TCA, CA or DCA was added, and the slices were harvested after 0, 5, 10, 15, 30 and 60 min. Concentration-dependent uptake was assessed by incubating slices with 0.02 to 2.00 mM bile acid for 10 min. The concentrations used were below the bile acid specific critical micellar concentration (CMC), which is approximately 13 mM for CA and 10 mM for both TCA and DCA [31]. After the incubation, the slices were washed three times with cold PBS and stored at -20 °C until bile acid measurement.

### Bile acid measurement

450 µl of 70 % methanol (v/v) was added to the slices and the slices were homogenized for 45 s using a Mini BeadBeater-8 (Biospec, USA). After centrifugation for 15 min at 13000 rpm (room temperature) 350 µl supernatant was transferred into a clean tube and dried by CentriVap Aqueous Concentrator System (Labconco, USA). The dry residue was reconstituted with 100 µl TRIS-HCl (50 mM, pH 7.6) and 2 µl was added to a 96-well plate. The total bile acid content was then analyzed using the DZ042A-K Total Bile Acids Test Kit.
The absorbance at 405 nm was measured every 5 min for 30 min with a Synergy HT Microplate Spectrophotometer (BioTek, USA), and the total bile acid content was calculated based on the calibrators and blank samples provided.

**Analysis of protein content**

The remaining pellet after homogenation of the slices for the ATP and bile acid measurement was dried overnight at 37°C. The dried pellet was dissolved in 200 µl NaOH (5 M) and incubated in water bath at 37 °C for 30 min. Afterwards 800 µl ultrapure water was added to a final concentration of 1 M NaOH and the sample was homogenized for 40 s. The amount of protein was determined using a DCTM Protein Assay (Bio-Rad, USA) with Lumicount microplate luminometer (Packard, USA). The measured amount of ATP and bile acids in the slices were corrected for the protein content.

**Statistical analysis**

Experiments were performed with intestinal tissues from at least three different humans and rats. For each condition within an experiment three slices were used and the average value of the three slices was used as the value for that particular condition of the individual human or rat. The results are given as the mean of individual experiments ± SEM. For the analysis of ATP content in rat and human PCIS, one-way ANOVA with a Bonferroni’s post-hoc test was used to compare multiple groups with one factor. The level of significance was set at p < 0.05. Kinetics parameters of ASBT were calculated and the Michaelis-Menten curves were fitted by GraphPadPrism 5 (GraphPad Inc., USA).

**RESULTS**

**Toxicity of bile acids**

As shown in Fig. 1, the ATP content decreased in a concentration-dependent manner in rat ileum slices incubated with DCA for 10 minutes. A significant decrease in ATP content was found in the slices incubated with 2.00 mM DCA (0.8 nmol/mg protein) compared to that in the control slices (3.8 nmol/mg protein), indicating that DCA at 2.00 mM decreased the viability of rat PCIS. In contrast, the ATP content in PCIS remained constant after incubation with 2 mM CA and TCA up to 1 h, indicating that these bile acids are not toxic under these circumstances. Human ileum slices incubated with 2.00 mM TCA for 3 h showed no significant decrease in ATP content compared to the control slices, 5.6 and 4.4 nmol/mg protein, respectively.
Fig. 1 ATP content in rat (n = 3) and human (n = 2) ileum PCIS after the indicated incubation time in absence or presence of CA, TCA, and DCA respectively. The data are depicted as mean ± SEM. One-way ANOVA was used for the comparison between each group incubated with bile acids and the corresponding control group. Statistical difference in intracellular ATP was found between the rat PCIS treated with 2.00 mM DCA and the corresponding control slices (p < 0.05).

Fig. 2 Morphological evaluation of rat ileum PCIS incubated in the absence or presence of CA, TCA and CA, respectively (0.04, 1.00 and 2.00 mM). No obvious decrease in morphological integrity is seen for sections a-j. In contrast sections k and l show flattening of the epithelial cells and discontinuous epithelial lining (indicated by arrows) indicating loss of viability of the enterocytes. Staining: hematoxylin and eosin. Scale bar is 200 µm.

For morphological evaluation rat PCIS were incubated in the same manner as the slices for the determination of intracellular ATP. In Fig. 2 morphological sections of slices incubated
with CA, TCA, and DCA, respectively, and stained with hematoxylin and eosin are depicted. Sections a-j show no obvious decrease in morphological integrity of the stroma, crypts, muscle layer and the epithelium. A continuous epithelial lining is present with cubic epithelial cells (Sections a-j). In contrast, in both sections k and l epithelial cells are flattened and a discontinuous epithelial lining is visible. A detachment of the muscle layer is seen in slices incubated with 1.00 mM DCA (Section k) and slices incubated with 2.00 mM DCA show a considerable loss of integrity of the epithelial lining (Section l). This morphological damage is in line with the decrease of intracellular ATP, confirming the loss of viability.

**Regional differences in uptake of TCA**

Uptake of TCA at a concentration of 0.04 mM was determined at different regions of rat and human intestine. This low concentration was chosen as pilot studies showed that no saturation of ASBT takes place at this concentration. The time-course of uptake in PCIS from different regions for rat and human intestine is shown in Fig. 3. Total uptake, i.e. both active transport and passive uptake of TCA at 37 °C was highest in the ileum for both rat and human whereas the passive uptake of TCA (Fig. 3 b and c) was relatively low and equal in all the intestinal regions of rat and human. The active uptake of TCA by ASBT was determined after subtraction of passive TCA uptake at 4 °C from the total uptake at 37 °C. As shown in Fig. 3c active uptake by rat ASBT only occurred in the ileum and not in the other regions. The same accounts for ASBT in human PCIS (Fig. 3 f). The uptake rate was linear during 15 minutes (Fig. 3c and f).

**Concentration-dependent uptake of bile acids**

Based on the results depicted in Fig. 3 c and f, the concentration-dependent ASBT-mediated uptake of TCA, DCA and CA was measured at 10 min with different concentrations ranging from 0.04 to 2.00 mM. The incubation time of 10 min was chosen since it was shown to be within the linear range of uptake, ensuring that the uptake rate was accurately measured [36]. Fig. 4 a and b show a concentration-dependent uptake of CA, TCA and DCA in rat PCIS at 4 and 37 °C, respectively. At 4 °C, bile acid uptake was lower and not saturable, indicating passive diffusion. The uptake of DCA at 4 °C was relatively high compared to the uptake of the less lipophilic CA and TCA. The rate of the active uptake was calculated and showed clear saturable uptake for all bile acids as depicted in Fig. 4 c. The apparent K_m of the rat ASBT for the three bile acids, calculated by fitting the data to the Michaelis-Menten equation, is shown in Fig. 4 c and listed in Table 2, and showed the following rank order: TCA < DCA < CA.
The apparent $V_{\text{max}}$ of rat ASBT, indicating the capacity of the ASBT-mediated uptake, is higher for DCA than for CA and TCA (Table 2) with the following rank order: DCA > TCA > CA (Fig. 4c). The contribution of rat ASBT to the total uptake was higher for TCA compared to CA and DCA at all concentrations (Fig. 4c), and was up to 87.0% at low concentrations of TCA and varied from 35.6% (DCA) to 56.4% (TCA) at 2.00 mM.

Fig. 3 Time-course of TCA uptake in rat and human PCIS incubated with 0.04 mM TCA. Panel a represents total uptake, i.e. passive and active uptake at 37 °C in rat duodenum (n = 4), jejunum (n = 6), ileum (n = 5) and colon (n = 4), while panel b shows the passive uptake at 4 °C in rat duodenum (n = 3), jejunum (n = 4), ileum (n = 4) and colon (n = 3). Active uptake in rat intestine is calculated by subtraction of passive uptake from total uptake and is depicted in panel c. Total uptake and passive uptake in the human intestine are depicted in d and e, respectively for jejunum (n = 4), ileum (n = 4), and colon (n = 3), and the resultant active uptake is shown in panel f. All values were corrected for the natural bile acid content in rat and human PCIS. Both in human and rat, the active uptake is present in the ileum only and is linear up to 15 min of incubation with TCA. Data are presented in mean ± SEM.

Human ileum is scarce and therefore only TCA was used for the characterization of the concentration-dependent uptake of ASBT. Total uptake and passive diffusion of TCA in human PCIS were depicted in Fig. 4d and e, respectively. Passive diffusion was determined using the average values of TCA uptake at 37 °C from human jejunum and colon, as these values were shown to be the same at 37 and 4 °C in these regions (see above, study on
regional differences), indicating that at 37 °C only passive diffusion takes place. Active uptake by human ASBT was shown to reach a plateau from 1.00 mM of TCA and the concentration-dependent uptake could be fitted according to the Michaelis-Menten equation (Fig. 4 f). The affinity of the human ASBT for TCA is lower than that of rat ASBT (K_m of 0.27 and 0.14 mM, respectively) but the capacity of TCA uptake is higher in human ileum (V_max in human ileum PCIS and rat ileum PCIS were 2.3 and 0.8 nmol/mg protein/min, respectively).

**Fig. 4** Concentration-dependent uptake of bile acids in rat and human ileum PCIS. CA, TCA and DCA (up to a concentration of 2.00 mM) were incubated for 10 min in rat PCIS while only TCA was used for human PCIS. Panel a represents the total uptake, i.e. passive and active uptake at 37 °C of CA (n = 6), TCA (n = 4) and DCA (n = 3) in rat ileum and panel b shows the passive uptake at 4 °C of CA (n = 6), TCA (n = 3) and DCA (n = 3). Active uptake of the three bile acids was calculated by subtraction of the passive uptake from the total uptake and is depicted in c. Total uptake (n = 4) and passive uptake (n = 4, see the note below) of TCA by human ileum PCIS is depicted in d and e, respectively. Active uptake in human ileum is shown in panel f. All values were corrected for the natural bile acid content of rat and human PCIS. Data are presented as mean ± SEM. The active uptake in c and f was fitted to the Michaelis-Menten equation. Note: Since passive diffusion was not measured in human intestinal slices, due to lack of sufficient slices, the values of passive diffusion in panel e was estimated by taking the average values from human jejunum and colon (n = 3 and 4, respectively) at 37 °C, where no active uptake was found (see Fig. 3).
DISCUSSION

In this study we investigated the applicability of rat and human PCIS as *ex vivo* models to study regional and species differences in bile acid uptake and ASBT-mediated transport. Therefore we first assessed the toxicity of ASBT substrates and bile acid uptake in different regions i.e. duodenum, jejunum, ileum and colon, of the rat intestine and jejunum, ileum and colon of the human intestine. ASBT-mediated transport was further characterized with respect to its kinetic parameters for three different bile acids in PCIS of the ileum. To the best of our knowledge, this is the first report on using PCIS as *ex vivo* model to study the ASBT-mediated bile acid uptake and showing for the first time the functional characteristics of the human ASBT *ex vivo*. In the present study TCA was used as a model conjugated bile acid to determine bile acid uptake along the intestine of both rat and human. As we expected, the uptake of TCA was high in rat and human ileum whereas little to no active uptake of TCA occurred in other regions (*Fig. 3 e and f*), in concordance with the exclusive localization of ASBT in the ileum [3, 6, 14]. Moreover, ASBT is very likely the key player in bile acid uptake along the intestine, as no other obvious active uptake was detectable in duodenum, jejunum and colon slices. This is in line with the observation, that *in vivo* the luminal bile acid concentration is decreased only to a small extent prior to the ileum [22, 37]. Besides ASBT, the organic anion transporting polypeptide (OATP) was reported as potential apical bile acid transporter *in vivo* and *in vitro*. Previous studies investigating expression of the members of OATP family have shown that both human OATP1A2 and rat Oatp1a5 are expressed at low levels on the apical membrane of the jejunum but they seem to be of minor importance for bile acid uptake [2, 8]. Moreover we found no active uptake of bile acids in the jejunum. Therefore, the influence of OATP on bile acid uptake in the ileum slices is probably very limited in our study. The organic solute transporter alpha-beta (OSTα-OSTβ) is also known to transport bile acids in the intestine. It is located in the basolateral membrane of the

<table>
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<th>Bile acid</th>
<th>$K_m^*$ [mM]</th>
<th>$V_{max}^*$ [nmol/mg protein/min]</th>
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<tbody>
<tr>
<td>Rat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA</td>
<td>0.98±0.69</td>
<td>0.80±0.26</td>
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<tr>
<td>TCA</td>
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<tr>
<td>DCA</td>
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<tr>
<td>TCA</td>
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<td>2.32±0.15</td>
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</table>

*Active transport parameters of ASBT are presented for the three bile acids in mean ± SEM.*
enterocytes and is involved in excretion of bile acids into the blood stream [2, 14, 38, 39]. In this study we have minimized the influence of efflux by OSTα-OSTβ by incubating the slices for only 10 min [36]. To confirm this, three experiments were performed to determine the efflux of TCA in bile acid free medium after incubation with 1.00 mM of TCA (data not shown). A maximal efflux rate of 0.20 nmol/mg protein/min was observed, which gradually decreased with a decreasing intracellular concentration of TCA. This indicates that its influence on the total uptake was low in PCIS under these experimental conditions. Co-incubation with a specific inhibitor of either ASBT or OSTα-OSTβ should be used in the future to accurately distinguish the contributions of these two transporters on bile acids accumulation. However, this approach is currently difficult due to the lack of truly specific inhibitors.

Subsequently, concentration-dependent uptake of CA, TCA and DCA was characterized in PCIS. At 4 °C only passive diffusion of the three bile acids can occur, which resulted in a linear increase of the uptake rate with increasing medium concentration of the bile acids. Passive diffusion of DCA was higher than that of CA and TCA. This was expected based on the different number of hydroxyl groups between the three bile acids which is inversely related to the rate of diffusion [40]. CA and TCA have three hydroxyl groups which make them more hydrophilic and less permeable to the cell membrane. However, a drawback of this method of assessing passive diffusion is that the altered membrane fluidity and molecule movement at 4 °C can influence passive diffusion [24, 35, 41]. Interestingly, the passive uptake at 4 °C was similar to the passive diffusion measured at 37 °C in human jejunum and colon (Fig. 3), suggesting that the influence of altered membrane fluidity was limited in PCIS. At concentrations higher than 1.00 mM uptake saturation was seen (Fig. 4 e and c), indicating that the uptake of bile acids was mediated by active transport in rat and human ileum. The rank order we found for the affinity of ASBT for bile acids (Table 2) is also seen in other studies where the highest affinity was found for conjugated bile acids [5, 11, 12, 15]. Discrepancies exist between studies regarding the affinity of ASBT for unconjugated bile acids in relation to the number of hydroxyl groups. Some studies indicate that the affinity is higher for dihydroxy bile acids than trihydroxy bile acids, while others found the opposite [11, 16, 23]. Based on the most recent review the affinity of ASBT for bile acids increases after glycine or taurine conjugation and in presence of fewer hydroxyl groups [22], which is in line with our findings regarding the rank order in affinity of ASBT for CA, TCA and DCA, respectively.

Our results also showed a different V\text{max} of rat ASBT for the three bile acids. A possible
explanation regarding this difference in $V_{\text{max}}$ is that bile acid binding to ASBT is a rate-limiting step which is faster when the affinity is higher [16]. It should be noted that the $V_{\text{max}}$ was determined at concentrations where DCA was shown to significantly decrease the viability of rat PCIS (Fig. 1 and Fig. 2 section l). DCA is known as a surface-active bile acid that can impair cell membranes and induce ROS formation which eventually can lead to triggering of apoptosis or necrosis [42-45]. Because of the toxicity of DCA at 2.00 mM the assessment of the $V_{\text{max}}$ of ASBT for DCA may be not accurate. However, uptake of DCA was larger compared to the uptake of TCA and CA also at the concentrations lower than 2.00 mM (Fig. 4), supporting the rank order of the $V_{\text{max}}$ for, DCA > TCA > CA. These results are in contrast with Schiff et al., who found the opposite rank order of $V_{\text{max}}$ of rat ASBT for CA, TCA and DCA [11]. A possible explanation is the difference in the settings of their model compared to our study, where they used an in situ perfusion model and measured mucosa-to-serosa flux rates.

In human PCIS we found that the capacity of ASBT for TCA is higher than that of rat ASBT. This may be explained by the higher abundance of ASBT in the human ileum that is required to reabsorb the much larger amounts of bile acids produced in human compared to rat. In previous studies the same trend is found for higher $V_{\text{max}}$ values for the human ASBT paralog Na+ -taurocholate co-transporting polypeptide (NTCP), indicating species differences in the expression of bile acid uptake transporters or bile acid binding as discussed previously [32, 46, 47]. However, the $K_{\text{m}}$ of human ASBT for TCA is higher compared to rat ASBT, which is probably related to the fact that TCA is more abundant in rat bile rather than in human bile.

In conclusion, this study shows that PCIS are a simple, reliable and fast ex vivo model to study passive uptake and ASBT-mediated transport of bile acids in the intestine of both rat and human. Also the toxicity of bile acids can be assessed with PCIS. ASBT activity was exclusively found in the ileum of both human and rat. The activity of ASBT-mediated bile acid uptake was characterized on ex vivo level, including the apparent $K_{\text{m}}$ and $V_{\text{max}}$. It was shown that the conjugated bile acid TCA had the highest affinity for ASBT, while the capacity of ASBT is the highest for the unconjugated DCA. Meanwhile the difference of ASBT activity between rat and human was evaluated, showing a higher activity in human. In the future PCIS can be to identify substrates and inhibitors of ASBT and to predict the extent of ASBT-mediated uptake in human PCIS.

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References


14. Alrefai Wa, Gill RK. Bile acid transporters: Structure, function, regulation and pathophysiological


29. van de Kerkhof EG, de Graaf IaM, Ungell A-IB, Groothuis GMM. Induction of Metabolism and Transport in Human Intestine: Validation of Precision-Cut Slices as a Tool to Study Induction of Drug Metabolism in Human Intestine in Vitro Drug Metabolism and Disposition 2008;36(3):604-13.


