Regional Distribution of Solute Carrier mRNA Expression Along the Human Intestinal Tract


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Received October 16, 2006; accepted January 11, 2007

ABSTRACT:

Intestinal absorption of drugs, nutrients, and other compounds is mediated by uptake transporters expressed at the apical enterocyte membrane. These compounds are returned to the intestinal lumen or released into portal circulation by intestinal efflux transporters expressed at apical or basolateral membranes, respectively. One important transporter superfamily, multiple members of which are intestinally expressed, are the solute carriers (SLCs). SLC expression levels may determine the pharmacokinetics of drugs that are substrates of these transporters. In this study we characterize the distribution of 15 human SLC transporter mRNAs in histologically normal biopsies from five regions of the intestine of 10 patients. The mRNA expression levels of CNT1, CNT2, apical sodium-dependent bile acid transporter (ABST), serotonin transporter (SERT), PEPT1, and OCTN2 exhibit marked differences between different regions of the intestine: the first five are predominantly expressed in the small intestine, whereas OCTN2 exhibits strongest expression in the colon. Two transporter mRNAs studied (OCTN1, OATP2B1) are expressed at similar levels in all gut sections. In addition, ENT2 mRNA is present at low levels across the colon, but not in the small intestine. The other six SLC mRNA s studied are not expressed in the intestine. Quantitative knowledge of transporter expression levels in different regions of the human gastrointestinal tract could be useful for designing intestinal delivery strategies for orally administered drugs. Furthermore, changes in transporter expression that occur in pathological states, such as inflammatory bowel disease, can now be defined more precisely by comparison with the expression levels measured in healthy individuals.

The members of the solute carrier (SLC) superfamily are membrane-associated transporters that facilitate the passage of solutes, including peptides, bile acids, and drugs, across cell membranes in epithelial tissues, such as intestine and liver (Hediger et al., 2004). In the intestine, SLCs are critically involved in drug absorption, thus determining distribution and pharmacokinetic characteristics of many drugs.

The human SLCs belong to 46 families (http://www.bioparadigm-s.org/slc/menu.asp) and are expressed in a polarized manner, either at the apical or basolateral cell membranes. The SLC28 and SLC29 families encode the high-affinity concentrative (CNT) and the low-affinity equilibrative (ENT) nucleoside transporters, respectively (Baldwin et al., 2004; Gray et al., 2004). Nucleoside transporters are essential for de novo nucleic acid synthesis, and for anticancer and antiviral therapy with nucleoside analogs. CNTs are mainly localized at the apical membranes of absorptive epithelia and mediate nucleoside uptake, whereas EN Ts are responsible for nucleoside efflux at basolateral membranes. Most transporters of the SLC22 family are polyspecific, transporting multiple different substrates, and are subdivided into three groups: organic cation transporters (OCTs), organic anion transporters (OATs), and organic zwitterion/cation transporters (OCTNs) (Koepsell and Endou, 2004). Two members of the last group, OCTN1 and OCTN2, have attracted much attention as polymorphisms, and the genes encoding them have been linked to inflammatory bowel disease (IBD) (Peltoketo et al., 2004). The SLCO family consists of organic anion-transporting polypeptides (OATPs), most of which have a broad substrate specificity (Hagenbuch and Meier, 2004). Although many OATPs play vital roles in the liver, some of them also have important functions in the intestine. A member of the SLC10 family, ASBT (apical sodium-dependent bile acid transporter; SLC10A2) is critical for enterohepatic circulation of...
bile acids by mediating their absorption at the apical membranes of enterocytes (Hagenbuch and Dawson, 2004). Reduced ASBT expression may cause decreased bile acid absorption and diarrhea in Crohn’s disease patients (Jung et al., 2004). Loss-of-function mutations in ASBT cause primary bile acid malabsorption, a rare intestinal disorder characterized by congenital diarrhea and reduced plasma cholesterol levels (Oelkers et al., 1997). The oligopeptide transporter PEPT1 (SLC15A1) at the apical membrane of intestinal epithelium mediates the uptake of nutrient-derived peptides, as well as of peptidomimetic drugs (Adibi, 2003). Colonic expression of PEPT1 is induced in patients suffering from IBD (Merlin et al., 2001), although it is unclear whether the aberrant PEPT1 expression is a cause or a consequence of inflammation. Serotonin transporter (SERT; SLC6A4) is a terminator of 5-hydroxytryptamine (5-HT) signaling, by mediating 5-HT reuptake at basolateral enterocyte membranes (Mawe et al., 2006). Reduced SERT expression is associated with ulcerative colitis and irritable bowel syndrome (IBS) (Coates et al., 2004). Furthermore, SERT polymorphisms are linked to IBS (Camilleri et al., 2002; Yeo et al., 2004).

Expression levels of SLTs may determine the degree of intestinal absorption of their transport substrates. Thus, knowledge about the distribution of SLTs along the intestine may assist in designing novel enterally delivered drugs. Relative SLC mRNA abundance along the human gut has been examined in two recent studies. Hruz et al. (2006) investigated ASBT expression in five intestinal regions, while Englund et al. (2006) studied the distribution of five SLTs (PEPT1, MCT1, OATP2B1, OCT1, OCTN2) in three gut segments. Here, we extend these analyses to 15 SLTs (CNT1/2, ENT1/2, OCTN1/2, OCT1, OAT2, OATP1A2/2B1/4A1, ASBT, SERT, PEPT1), using histologically normal biopsies from five intestinal segments obtained from 10 individuals.

Materials and Methods

Patients. The study was approved by the ethical committee of the University Hospital Zurich (EK-837) and informed consent was obtained from all patients. Histologically normal biopsies from five intestinal sections, namely, duodenum, ileum, colon ascendens, colon transversum, and colon descendens, were obtained from 10 white subjects undergoing upper and lower intestinal endoscopy. Information on each patient (age, gender, reason for endoscopy, drug intake, additional diagnoses) is shown in Table 1.

RNA Isolation, Reverse Transcription, and Real-Time PCR. Biopsies were rinsed in phosphate-buffered saline, transferred to 1 ml of TRIzol (Invitrogen, Basel, Switzerland), and disintegrated by repeated syringing through 21 gauge needles. After isolation of RNAs, their concentrations were determined using NanoDrop ND-1000 (Witec AG, Littau, Switzerland). The A260/A280 ratios of all RNAs were 1.9 to 2.1. Then, 3 μg of each RNA were reverse-transcribed by random priming in 40-μl reactions (Reverse Transcription System; Promega, Madison, Wisconsin, USA), diluted to a volume of 150 μl with nuclelease-free water. Real-time PCR was performed on an ABI PRISM 7700 sequence detection system (Applied Biosystems, Foster City, CA) using 5 μl of cDNA, 1.25 μl of TaqMan Gene Expression Assays (Applied Biosystems; Supplemental Data Table 1) and 12.5 μl of 2X Universal PCR MasterMix (Applied Biosystems) in a final reaction volume of 25 μl. The cycling conditions were: stage 1 (50°C, 2 min); stage 2 (95°C, 10 min); stage 3, 40 times (95°C, 15 s, followed by 60°C, 1 min). Each PCR was performed in triplicate, and the standard deviations of all triplicates were less than 7%. For each TaqMan assay, a dilution series (30, 300, 3000, 30,000, 300,000, and 3,000,000 copies) of the corresponding cloned PCR product (see below) was included on each PCR plate, to generate standard curves for absolute quantification of cDNA copy numbers. Data are expressed as SLC cDNA copy numbers in relation to villin cDNA copy numbers. This method assumes that the reverse transcription efficiency of all mRNAs is comparable.

Cloning of PCR Products. Before real-time PCR, preparative PCRs using TaqMan Gene Expression assays and Human Universal QUICK-Clone cDNA (Takara Bio Europe, Saint-Germain-en-Laye, France) as a template were performed. PCR products were cloned into the pCR2 vector (Invitrogen) or, in the case of OCTN2, into the pGEM-T vector (Promega, Bad Bialystok, Switzerland), quantitated with NanoDrop ND-1000, and diluted as described above.

Statistical Analysis. The mRNA expression levels of SLC genes between different gut sections were analyzed by one-way analysis of variance, followed by post hoc analysis with Tukey’s test (Prism; GraphPad Software Inc., San Diego, CA). p values less than 0.05 were considered significant.

Results

Relative Abundance of Different SLC mRNAs within Intestinal Sections. The mRNA levels of 15 SLCs in five different intestinal regions were measured by real-time PCR and expressed as copy numbers of SLC cDNA in relation to copy numbers of villin cDNA. Villin is commonly used as the reference gene for epithelial content in intestinal samples (Zimmermann et al., 2005; Hruz et al., 2006). We note that, in agreement with the study by Englund et al. (2006), in our patient group villin mRNA expression was slightly higher in duodenum than in colon segments (colon ascendens, p < 0.01; colon transversum, p < 0.05; colon descendens, p < 0.01), possibly causing a slight bias toward low duodenal SLC expression. In contrast to the
SCLs are shown in descending order according to their relative mRNA expression levels in different intestinal sections. SLCs with relative mean mRNA expression levels >0.01, and thus considered detectable, are highlighted in bold. The corresponding median values obtained from the 10 patients are given in parentheses for the detectable SLC mRNAs.

**Discussion**

We have quantified the regional mRNA expression of 15 SLC genes along the human intestinal tract, using histologically normal tissue derived from duodenum, ileum, colon ascendens, colon transversum, and colon descendens. We found marked differences in SLC mRNA levels between intestinal sections. Nine SLC mRNAs were detectable in at least one section of the intestine. Among these, we observed three different expression patterns: 1) SLCs predominantly or exclusively expressed in the duodenum and ileum (CNT1, CNT2, ASBT, SERT, PEPT1), 2) SLCs expressed in all five intestinal sections at similar levels (OCTN1, OATP2B1), and 3) SLCs expressed at a higher level in colon than in the small intestine (OCTN2, ENT2). None of the SLC mRNAs exhibited significantly different expression levels among the three colon segments. Six SLC mRNAs were not detectable in any intestinal section (CNT3, ENT1, OCT1, OAT2, OATP1A2, and OATP4A1).

Our results are in agreement with recent studies showing that the OCT1 and OAT2 mRNAs are either absent or expressed at very low levels in the human colon (Englund et al., 2006; Seithel et al., 2006).

It is important to note that the mRNA levels may not reflect the exact amount of the SLC protein or functional activity of a transporter, which may be subject to post-transcriptional regulation. However, obtaining sufficient amounts of intestinal tissue from healthy individuals to allow protein extraction or functional studies, in addition to RNA isolation, for all 15 SLCs studied here is not feasible. However, we believe that quantitation of mRNA levels provides a relevant indication of the abundance of SLC transporters in specific gut sections.

For those SLC mRNAs that were detectable, our analysis of CNT1,
CNT2, SERT, OCTN1, and ENT2 expression is the first systematic study of their regional distribution in the human intestine. Our data pertaining to ASBT, PEPT1, and OATP2B1 mRNA distribution are in agreement with two recent studies that also investigated the relative mRNA levels of subsets of SLC genes along the human intestine (Englund et al., 2006; Hruz et al., 2006). There is one difference between our results and those obtained by Englund et al. (2006): in our study OCTN2 mRNA expression was similar between duodenum and ileum, whereas Englund et al. (2006) reported that OCTN2 expression level is higher in the ileum, compared with duodenum. The reason for this discrepancy is unclear at present.

We observed a high degree of interindividual variability in the expression of SLC mRNAs in the intestine. This may result from differences in intestinal metabolic microenvironment between patients, caused by genetic, hormonal, and dietary variations, or from differential exposure to drugs and environmental toxins. All these factors may affect transcriptional rates of specific SLC genes, thus causing transient or long-term changes in mRNA levels. Transcriptional regulatory mechanisms have been studied for a few of the SLC genes investigated in this study. Our current approach may provide physiological support for such molecular studies, or suggest novel pathways by which the SLC genes are regulated. For example, consistent with the proposal that the human ASBT promoter is activated by the glucocorticoid receptor (Jung et al., 2004), ileal ASBT mRNA expression is particularly high in patient 4 (Fig. 1C), receiving glucocorticoid therapy (Table 1). Interestingly, OATP2B1 expression is similarly elevated in the ileum and colon of patient 4 (Fig. 1H), suggesting that the OATP2B1 gene may also be activated by glucocorticoids. Duodenal expression of six SLCs (CNT1, CNT2, SERT, PEPT1, OCTN2, OATP2B1) in patient 7 was clearly above the mean level derived from all 10 patients (Fig. 1A, B, D, E, G, and H). This patient had been treated with carbamazepine (Table 1), a known agonist of the transcriptional regulator of drug transport and detoxification pathways, pregnane X receptor (Luo et al., 2002). It will be interesting to study whether the SLCs highly expressed in patient 7 might thus be coordinately regulated by pregnane X receptor.

We did not observe statistically significant gender differences in SLC expression. However, to exclude sex-dependent effects on SLC expression, the patient cohort should be large enough to allow division of female subjects into pre- or postmenopausal ones, and those who are, or are not, taking hormone-containing contraceptives.

The patients in this study showed no apparent signs of intestinal diseases. Results obtained in healthy subjects form a basis for understanding changes in SLC gene expression in intestinal diseases, and
may help in adapting therapeutic interventions in such pathologies. Changes in SLC expression occur in IBD, although it remains unclear whether these are consequences of the inflammatory pathways or causative factors in the pathogenic process, or both. For example, PEPT1 is normally expressed at the apical membrane of the enterocytes of the small intestine, but has been reported to be abnormally expressed in the colon in states of chronic inflammation (Merlin et al., 2001). SERT mRNA expression is reduced in patients suffering from IBS and ulcerative colitis (Coates et al., 2004), implying that mucosal 5-HT uptake is impaired in these diseases. Variants of the OCTN1 and OCTN2 genes are linked to Crohn’s disease (Peltekova et al., 2004), and OCTN1 and OCTN2 expression levels may also be associated with intestinal inflammation.

Intestinal drug absorption determines drug efficacy and pharmacokinetics. Several SLCs in this study mediate enteral uptake of specific drugs. For example, nucleoside analogues used in antiviral therapy are absorbed via CNT1 and CNT2. ASBT is an efficient uptake system for bile acids but has not been shown to transport drugs. However, it is an attractive target for delivery of prodrugs designed as bile acid derivatives (Balakrishnan and Polli, 2006). Determining regional expression levels of drug and prodrug transporters provides indications about the site of intestinal absorption of a drug. This may help in designing enterally delivered drugs with more accurate absorption properties and more predictable pharmacokinetic characteristics.

Acknowledgments. We thank M. Keller for kind assistance in obtaining biopsies.

References


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