ABSTRACT:

Efflux transporters such as P-glycoprotein and multidrug resistance-associated proteins (MRPs) in the intestinal wall restrict intestinal drug transport. To overcome this limitation for enteral drug absorption, galenical targeting approaches have been proposed for site-specific luminal drug release in segments of the gut, where expression of the respective absorption-limiting transporter is minimal. Therefore, expression of multidrug resistance gene 1 (MDR1) and MRP1-5 was systematically investigated in 10 healthy subjects. Biopsies were taken from different segments of the gastrointestinal tract (from duodenum and terminal ileum, as well as ascending, transverse, descending, and sigmoid colon). Gene expression was investigated by quantitative real-time PCR (TaqMan). MRP3 appeared to be the most abundantly expressed transporter in investigated parts of the human intestine, except for the terminal ileum, where MDR1 showed the highest expression. The ranking of transporter gene expression in the duodenum was MRP3 > MDR1 > MRP2 > MRP5 > MRP4 > MRP1. In the terminal ileum, the ranking order was as follows: MDR1 > MRP3 > MRP1 > MRP5 > MRP4 > MRP2. In all segments of the colon (ascending, transverse, descending, and sigmoid colon), the transporter gene expression showed the following order: MRP3 > MDR1 > MRP4 > MRP5 > MRP1 > MRP2. We have shown, for the first time, systematic site-specific expression of MDR1 and MRP mRNA along the gastrointestinal tract in humans. All transporters showed alterations in their expression levels from the duodenum to sigmoid colon. The most pronounced changes were observed for MRP2, with high levels in the small intestine and hardly any expression in colonic segments. This knowledge may be useful to develop new targeting strategies for enteral drug delivery.

Efflux transporters in the intestinal wall form a barrier to cellular accumulation of toxins as well as to drug absorption (Schinkel, 1997). Important efflux proteins in the gut are P-glycoprotein [gene product of the multidrug resistance 1 (MDR1) gene] and multidrug resistance-associated protein (MRP) transporters. They belong to the superfamily of ATP-binding cassette (ABC) transporters. ABC transporters mediate the translocation of a wide variety of substances across cellular membranes using ATP hydrolysis (Horio et al., 1988; Senior et al., 1995). The expression of ABC transporter genes is widespread throughout many tissues, most notably in excretory sites such as the liver, kidney, blood-brain barrier, and intestine. Therefore, they play a critical role in absorption and tissue distribution of orally administered drugs (Schuetz et al., 1998; Ambudkar et al., 1999). Due to their broad substrate specificity, they may influence the pharmacokinetics of many chemically unrelated substances (e.g., HIV drugs, anticancer drugs, endogenous compounds) (Lee et al., 1997; Schinkel, 1998; Schuetz et al., 1999; Borst et al., 2000). MDR1 preferentially extrudes large hydrophobic, positively charged molecules, whereas the members of the MRP family extrude both hydrophobic uncharged molecules and water-soluble anionic compounds.

There is little knowledge about the expression pattern of those ABC transporters along the human intestine. Taipalensuu et al. (2001) investigated gene expression of 10 ABC transporters in jejunal biopsies from healthy subjects. The highest expression was shown for breast cancer resistance protein and MRP2. Nakamura et al. (2002) investigated the expression of three ABC transporters in duodenal and colorectal tissues in humans. In comparison to duodenum, in colon they found a decrease in MDR1 expression, equal levels of MRP1, and a strong decrease in MRP2 expression. However, this comparison was not obtained in the same subjects. Therefore, the intraindividual expression differences between these transporters could not be assessed.

Knowledge of the topographical distribution may be important for the development of specific galenical targeting approaches, which may be utilized to improve intestinal absorption of drugs. Therefore, in this study, the expression of MDR1 and MRP1-5 genes was investigated in the human intestine of 10 healthy subjects.

Materials and Methods

Intestinal biopsies were obtained from a group of 10 healthy subjects (5 female, 5 male, aged 50–76 years, average age 62 years, no medication), which served as a control group in a clinical study designed to investigate the regional expression of different genes in patients with inflammatory bowel disease. The study protocol included specifically the investigation of drug-transporting proteins and was approved by the local ethical committee. Informed consent

ABBREVIATIONS: MDR1, multidrug resistance gene 1; MRP1-5, multidrug resistance-associated protein isoforms 1 to 5; PCR, polymerase chain reaction; ABC, ATP-binding cassette; SN-38, 7-ethyl-10-hydroxycamptothecin (active metabolite of irinotecan).
DNase I digestion (Invitrogen, Basel, Switzerland), and RNA was extracted using the RNeasy Mini Kit (QIAGEN GmbH, Hilden, Germany). For RNA isolation, two biopsies from each intestinal region were collected in a tube with RNAlater (Ambion, Austin, TX) and stored at -80°C. For duodenal samples, the amount of transporter mRNA was below the detection limit in four biopsies, which is equivalent to one subject having to be discarded, leading to the exclusion of nine duodenal samples. Due to low enterocyte content, duodenal biopsies from one subject had to be discarded, leading to the exclusion of nine duodenal samples.

Preparation of Samples. The samples were immediately submerged in a tube with RNAlater (Ambion, Austin, TX) and stored at -80°C until further processing. For RNA isolation, two biopsies from each intestinal tissue were homogenized for 30 s (Polytron PT 2100; Kinematica AG, Littau, Switzerland) and RNA was extracted using the RNeasy Mini Kit (QIAGEN GmbH, Hilden, Germany) following the instructions provided by the manufacturer. RNA was quantified with a GeneQuant photometer (Pilter, Inc., Taby, Sweden). After DNase I digestion (Invitrogen, Basel, Switzerland), 1.5 μg of total RNA was reverse-transcribed by SuperScript (Invitrogen) according to the manufacturer’s protocol, using random hexamers as primers.

TaqMan analysis was carried out on a 7900HT Sequence Detection System (Applied Biosystems, Rotkreuz, Switzerland). PCR conditions were 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. Each TaqMan reaction contained 25 ng of cDNA in a total volume of 25 μL. TaqMan Universal PCR Mastermix from Applied Biosystems was used. The concentrations of primers and probes were 900 nM and 225 nM, respectively. Primers and probes were designed according to the guidelines of Applied Biosystems with help of Primer Express 2.0 software (Table 1). Primers were synthesized by Invitrogen (Basel, Switzerland) and probes by Eurogentec (Seraing, Belgium). For absolute mRNA quantification, we used external standard curves. The standards were reverse-transcription-PCR products of the appropriate gene (Table 1). These cDNA standards were purified by running a 1.5% agarose gel and by a subsequent gel extraction (gel extraction kit, QIAGEN GmbH). They were quantified using the PicoGreen reagent (Molecular Probes, Eugene, OR) and were checked by sequencing (Microsynth GmbH, Balgach, Switzerland).

Table 1: Primers and probes for TaqMan Analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR1 (ABCB1)</td>
<td>5'-AGCCATGTTCTGTCGAAGAGCTCCACATGCTC-3'</td>
<td>5'-CTGTACGCAAGAGAGCTCCACATGCTC-3'</td>
</tr>
<tr>
<td>MRP1 (ABCC1)</td>
<td>5'-CTGCCAGCTTCTCTTCTCTCTCTCTCTC-3'</td>
<td>5'-CTGCCAGCTTCTCTCTCTCTCTCTCTC-3'</td>
</tr>
<tr>
<td>MRP2 (ABCC2)</td>
<td>5'-CTGCCAGCTTCTCTCTCTCTCTCTCTC-3'</td>
<td>5'-CTGCCAGCTTCTCTCTCTCTCTCTCTC-3'</td>
</tr>
<tr>
<td>MRP3 (ABCC3)</td>
<td>5'-CTGCCAGCTTCTCTCTCTCTCTCTCTC-3'</td>
<td>5'-CTGCCAGCTTCTCTCTCTCTCTCTCTC-3'</td>
</tr>
<tr>
<td>MRP4 (ABCC4)</td>
<td>5'-CTGCCAGCTTCTCTCTCTCTCTCTCTC-3'</td>
<td>5'-CTGCCAGCTTCTCTCTCTCTCTCTCTC-3'</td>
</tr>
<tr>
<td>MRP5 (ABCC5)</td>
<td>5'-CTGCCAGCTTCTCTCTCTCTCTCTCTC-3'</td>
<td>5'-CTGCCAGCTTCTCTCTCTCTCTCTCTC-3'</td>
</tr>
<tr>
<td>Villin (VIL1)</td>
<td>5'-CTGCCAGCTTCTCTCTCTCTCTCTCTC-3'</td>
<td>5'-CTGCCAGCTTCTCTCTCTCTCTCTCTC-3'</td>
</tr>
</tbody>
</table>

The standards were reverse-transcription-PCR products of the appropriate gene (Table 1). These cDNA standards were purified by running a 1.5% agarose gel and by a subsequent gel extraction (gel extraction kit, QIAGEN GmbH). They were quantified using the PicoGreen reagent (Molecular Probes, Eugene, OR) and were checked by sequencing (Microsynth GmbH, Balgach, Switzerland). All samples were run in triplicates, and non-reverse-transcribed RNA served as a negative control.

For each sample, the number of transporter transcripts (MDR1, MRP1–5) and the number of villin transcripts were determined. By calculating the ratio of transporter/villin mRNA, the transporter expression was normalized. Enterocytes represent only a small fraction of the cells obtained in an intestinal biopsy. Determination of villin, an enterocyte-specific, constitutively expressed protein, can be used to control for the variation of enterocyte content in biopsy (Lown et al., 1994). Therefore, transporter mRNA concentrations were expressed as a ratio with the villin levels of the same sample. These villin-corrected values provide a relative measure of enterocyte concentration (Lown et al., 1997). Results with this approach have already been published (Taipalesen et al., 2001; Mouly and Paine, 2003).

Statistical Analysis. Gene expression was compared between the different intestinal segments by analysis of variance. In the case of significant differences, all segments were compared with the expression in duodenum using two-sided Dunnett’s multicomparison test. The level of significance was P < 0.05. Comparisons were performed using SPSS for Windows software (version 11.0; SPSS Inc., Chicago, IL).

Results. There was a considerable interindividual variability of transporter gene expression amounting on average to 34% (CV%). Figure 1 displays the expression and ranking of all transporters in the analyzed tissues normalized to villin. MRP3 appeared to be the most abundantly expressed transporter in the investigated parts of the human intestine, except for the terminal ileum, where MDR1 showed the highest expression. The ranking of transporter gene expression in the duodenum was MRP3 > MDR1 > MRP2 > MRP5 > MRP4 > MRP1. In the terminal ileum the ranking order was as follows:
MDR1 > MRP3 >> MRP1 = MRP5 = MRP4 > MRP2. In all segments of the colon (ascending, transverse, descending, and sigmoid colon), the transporter expression showed the following order: MRP3 >> MDR1 > MRP4 = MRP5 > MRP1 >> MRP2.

Figure 2 shows the expression pattern of each individual transporter from the duodenum to the sigmoid colon normalized to villin. Compared with the duodenum, the expression of MDR1 was 4-fold higher in the terminal ileum and approximately 2-fold higher in the colonic segments. MRP1 exhibited a 2- to 3-fold higher expression in both the terminal ileum and colon compared with duodenum. MRP2 showed highest expression in the duodenum, half-levels in the terminal ileum, and hardly any MRP2 transcripts in each colonic segment. MRP3, MRP4, and MRP5 exhibited a similar expression pattern with equal levels in the duodenum and terminal ileum, but a 2- to 3-fold increase in the colon. Within the colon, MRP1, MRP3, and MRP5 showed an expression pattern with decreasing levels from proximal to distal, whereas MDR1, MRP2, and MRP4 levels remained rather constant.

Discussion

Only little information is available about the expression of ABC transporters along the intestinal tract. Available information relates mainly to MDR1 and MRP2 expression (Dietrich et al., 2003; Lindell et al., 2003). Furthermore, previous studies have focused on isolated parts of the intestine (Taipalensuu et al., 2001; Lindell et al., 2003), on animal models (Achira et al., 2002; Takara et al., 2003), or on cancer cells (Nakamura et al., 2002; Li et al., 2003; Pfrender et al., 2003). Here, we present a systematic investigation of multidrug resistance protein mRNA expression in various parts of the human intestine from proximal to distal within the same subject. One drawback of the study is the lack of samples from the jejunum, an important site for drug absorption. The subjects in our study underwent combined gastroscopy and colonoscopy procedures for screening of gastrointestinal cancer. Therefore, an additional jejunoscopy was not performed. However, Taipalensuu et al. (2001) focused on the human jejunum and found a transporter expression with the following ranking: MRP2 > MDR1 > MRP5 > MRP4 = MRP1 > MRP3. Besides the high MRP2 levels, the transporter expression pattern in the jejunum shows strong similarity to the pattern we found in the terminal ileum, which is conclusive because of the proximity of these tissues.

It is suggested that MDR1 physiologically functions as a gatekeeper against xenobiotics in the gut. The bioavailability of many drugs is reduced due to MDR1 efflux. MDR1 shows an extremely broad substrate specificity, including anticancer agents, antibiotics, antivirals, calcium channel blockers, and immunosuppressants. With respect to the expression of MDR1 in the human intestine, an increase from proximal to distal was stated, with the highest expression levels documented in the colon (Fricker et al., 1996; Dietrich et al., 2003; Chan et al., 2004). In mice, however, Chianale et al. (1995) found the highest levels of mdr3 mRNA in the ileum. In the rat intestine, the P-glycoprotein-mediated drug efflux showed highest activity in the ileum as well (Stephens et al., 2001). We could also demonstrate, in humans, higher MDR1 mRNA levels in the terminal ileum compared with the duodenum. These results are consistent with human data from Moully and Pain (2003), who reported an increase in P-glycoprotein from duodenum to ileum. Additionally, our results indicate the highest MDR1 expression in the terminal ileum within the investigated segments of the human intestine. It appeared to be 4-fold higher in the terminal ileum compared with the duodenum and 2-fold higher compared with the colon. Moreover, MDR1 was the most abundantly expressed transporter in the terminal ileum compared with all other ABC transporters that were analyzed in this study.

MRP1 showed the lowest variation in mRNA levels within the intestinal tract. This is in good agreement with the fact that MRP1 is expressed ubiquitously. Physiologically important substrates for MRP1 include glutathione S-conjugates such as leukotriene C4, as well as bilirubin glucuronides (Kepler et al., 1998). In addition, anionic drugs and drugs conjugated to glutathione, like methotrexate or arsenite, are also transported by MRP1 (Bakos et al., 2000; Vernhet et al., 2000).

A previous study revealed that MRP2 is the ABC transporter with...
the highest expression besides breast cancer resistance protein in the human jejunum (Taipalensuu et al., 2001). We found relatively low MRP2 levels in the human duodenum and even lower levels in the terminal ileum, but almost no MRP2 expression in the entire colon. These results were also found in the rat intestine (Mottino et al., 2000; Rost et al., 2002), but up to now, they were not confirmed in humans. The results are also consistent with the expression pattern of glutathione S-transferase in the human gastrointestinal tract mucosa (Coles et al., 2002). This phase II metabolizing enzyme provides the conjugated compounds for subsequent export by MRP2 or MRP1. The substrate specificity of MRP2 is similar to that of MRP1, and includes glutathione conjugates, bilirubin glucuronides, and a number of drugs and their conjugated drug metabolites (Jedlitschky et al., 1997; Kawabe et al., 1999). These drugs include pravastatin, temocaprilat, irinotecan, SN-38, arsenite, cisplatin, methotrexate, vincristine, saquinavir, and ceftriaxone (Kusuhara and Sugiyama, 2002; Dietrich et al., 2003). Regarding the amount of drugs transported by MRP2, a drug targeting which
Acknowledgments. We thank Ursula Behrens for excellent technical assistance. We thank the nurses of the Department of Gastroenterology for excellent technical assistance during biopsies.

References


Address correspondence to: Dr. Juergen Drewe, Clinic for Pharmacology & Toxicology, University Clinic Basel/Universitaetsspital, Hebelstr. 2, CH-4031 Basel, Switzerland. E-mail: juergen.drewe@unibas.ch.