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.

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 intragroup 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
was obtained from all subjects prior to inclusion. No macroscopically pathological findings were observed during endoscopies in these subjects. Three to four biopsies were obtained from duodenum, terminal ileum, ascending colon, transverse colon, descending colon, and sigmoid colon. Due to low enteroocyte content, duodenal biopsies from one subject had to be discarded, leading to 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 region were homogenized for 30 s (Polytron PT 2100; Kinematika 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 (Pfizer, Inc., Ta ¨by, Sweden). After digestion of RNA, the samples were quantified using the PicoGreen reagent (Molecular Probes, Eugene, OR) and RNA was stored at –80°C. The standards were reverse transcription-PCR products of the appropriate gene (Table 1). These cDNA standards were quantified using the PicoGreen reagent (Molecular Probes, Eugene, OR) and were checked by sequencing (Microsynth GmbH, Balgach, Switzerland).

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<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
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<tbody>
<tr>
<td>MDR1 (ABCB1)</td>
<td>5’-ACAGTCCCAGTGAATCGAGC-3’</td>
<td>5’-CTTTATTGAGGCGCTTACCCG-3’</td>
</tr>
<tr>
<td>MR1 (ABCC1)</td>
<td>5’-AGGTATTGACACCTGAGTCG-3’</td>
<td>5’-TATACAGTCGAGTGCG-3’</td>
</tr>
<tr>
<td>MR2 (ABCC2)</td>
<td>5’-AGTATCCGAGCTTACCCGAGA-3’</td>
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<tr>
<td>MR3 (ABCC3)</td>
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<tr>
<td>MRP4 (ABCC4)</td>
<td>5’-CTAGAGGACTTCCTGAGAGA-3’</td>
<td>5’-AAATGCTATTTTCAAGATCCG-3’</td>
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<tr>
<td>MRP5 (ABCC5)</td>
<td>5’-AGAGAGACCCAAATGAAAGACA-3’</td>
<td>5’-ATGAGATGAGTGAGTGAGTGAG-3’</td>
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<tr>
<td>MRP5 (ABCC5)</td>
<td>5’-AGAGAGACCCAAATGAAAGACA-3’</td>
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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 > MR1. In the terminal ileum the ranking order was as follows:
MDR1 \(\gg\) MRP3 \(\approx\) MRP1 \(\approx\) MRP5 \(\approx\) MRP4 \(>\) MRP2. In all segments of the colon (ascending, transverse, descending, and sigmoid colon), the transporter expression showed the following order: MRP3 \(\gg\) MDR1 \(\approx\) MRP4 \(\approx\) MRP5 \(>\) MRP1 \(\gg\) 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; Pfrunder 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 \(\approx\) MRP3 \(>\) MRP5 \(\approx\) MRP1 \(>\) MRP4. 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 Mouth 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

![Graphs showing transporter-specific gene expression in different gut segments normalized to villin expression.](image-url)
circumvents absorption sites with high MRP2 expression would be of benefit, especially for drugs with low bioavailability.

MRP3 transports a wide range of bile salts and seems to be involved in their reabsorption (Hirohashi et al., 2000). MRP3 transcription of cell lines conferred resistance to epipodophyllotoxins, vincristine and methotrexate (Kool et al., 1999). For MRP3, Rost et al. (2002) showed low expression in the rat duodenum and high expression in the ileum and colon. Our human data indicate low MRP3 levels in the duodenum as well as in the terminal ileum but also high expression in the colon. Within the colon, MRP3 expression diminished slightly from proximal to distal segments. This reduction in transporter expression from ascending to sigmoid colon was observed for MRP1, MRP3, and MRP5. Interestingly, all of these transporters are located on the basolateral membrane. For MDR1, MRP2, and MRP4, probably located on the apical membrane (Chan et al., 2004), we observed rather constant expression levels throughout the entire colon.

With respect to MRP4, we found equal expression levels in the duodenum and the terminal ileum but a 3-fold increase in the colon. To our knowledge, there is no previous publication on the MRP4 expression in the colon. The significance of MRP4 in drug transport is at present unclear. However, an overexpression of MRP4 severely impaired the antiviral efficacy of adefovir, azidothymidine, and other nucleoside analogs in cell lines (Schuetz et al., 1999). Other substrates include folic acid, bile acids, methotrexate, and 6-mercaptourine (Wielinga et al., 2002; Chan et al., 2004). A physiological role of MRP4 might be the release of prostaglandins from cells (Chan et al., 2004).

MRP5 expression appeared to be concordant to MRP4 expression with low levels in the duodenum and the terminal ileum, but a 2-fold increase in the different colon segments. Both transporters have an affinity to nucleotide-based substrates. There are no reports, at present, which could suggest a role for MRP5 in intestinal drug disposition. Experiments with transfected cells showed enhanced efflux of 2,4-dinitrophenyl-5-glucuronic acid, adefovir, and the purine analogs 6-mercaptopurine and thioguanine (Wijnholds et al., 2000).

Jedlitschky et al. (2000) demonstrated that MRP5 transports the cyclic nucleotides cAMP and cGMP, but the physiological function of this transporter remains to be elucidated.

Although our results indicate significant changes of MDR1 and MRP1-5 gene expression in investigated parts of the human intestine, this does not necessarily correlate with protein expression or function. Additional studies regarding the effect of expression on protein levels are therefore required. The impact of these transporters should be evaluated for drug permeating epithelial barriers, especially during pharmacological development of novel classes of therapeutic compounds. Selectivity of inhibitors, in particular, for human efflux transporters located at the apical mucosal membrane (such as MDR1, MRP2, and MRP4), remains to be examined, and further studies are required. Therefore, the knowledge of the transporter expression throughout the human intestine might be of special value.

Conclusion

We have shown, for the first time, systematic site-specific expression of MDR1 and MRP isoforms along the gastrointestinal tract in humans. All transporters showed alterations in their expression levels from the duodenum to the sigmoid colon. The most pronounced changes were observed for MRP2, with high levels in the small intestine and hardly any expression in coli segments. This knowledge may be useful to develop new targeting strategies for enteral drug delivery.

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References


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