Correlation of Gene Expression of Ten Drug Efflux Proteins of the ATP-Binding Cassette Transporter Family in Normal Human Jejunum and in Human Intestinal Epithelial Caco-2 Cell Monolayers

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ABSTRACT

This investigation describes the expression and interindividual variability in transcript levels of multiple drug efflux systems in the human jejunum and compares the expression profiles in these cells with that of the commonly used Caco-2 cell drug absorption model. Transcript levels of ten-drug efflux proteins of the ATP-binding cassette (ABC) transporter family [MDR1, MDR3, ABCB5, MRPs 1–6, and breast cancer resistance protein (BCRP)], lung resistance-related protein (LRP), and CYP3A4 were determined using quantitative polymerase chain reaction in jejunal biopsies from 13 healthy human subjects and in Caco-2 cells. All genes except ABCB5 were expressed, and transcript levels varied between individuals only by a factor of 2 to 3. Surprisingly, BCRP and MRP2 transcripts were more abundant in jejunum than MDR1 transcripts. Jejunal transcript levels of the different ABC transporters spanned a range of three log units with the rank order: BCRP ≈ MRP2 > MDR1 ≈ MRPs 3–6 > MRP1 > MRP4 > MDR3. Furthermore, transcript levels of 9 of 10 ABC transporters correlated well between jejunum and Caco-2 cells ($r^2 = 0.90$). However, BCRP exhibited a 100-fold lower transcript level in Caco-2 cells compared with jejunum. Thus, the expression of a number of efflux protein transcripts in jejunum are equal to, or even higher than, that of MDR1, suggesting that the roles of these proteins (in particular BCRP and MRP2) in intestinal drug efflux have been underestimated. Also, we tentatively conclude that the Caco-2 cell line is a useful model of jejunal drug efflux, if the low expression of BCRP is taken into account.

Apart from the multidrug resistance protein MDR1, many other recently discovered efflux proteins of the ATP-binding cassette (ABC) transporter superfamily may influence the pharmacokinetics, tissue distribution, and pharmacodynamics of drugs (Wacher et al., 1998; Ambudkar et al., 1999; Borst et al., 2000; Jonker et al., 2000). These more recent discoveries include at least six different multidrug resistance-associated proteins (MRPs) (Borst et al., 2000) as well as breast cancer resistance protein (BCRP) (Doyle et al., 1998). Also, a large number of ABC transporters are mainly known as expressed sequence tags and have been assigned to different subfamilies according to their similarity to known ABC transporters. One of these orphan ABC transporters, ABCB5, was included in this study because of its similarity to MDR1. The tissue expression pattern and the transcript size of ABCB5 have led to it being described as a housekeeping (expressed in all tissues) full-molecule (two transmembrane and two ATP-binding domains) ABC transporter (Allikmets et al., 1996). Because of the broad substrate specificities of these transporters, drugs of many pharmacological classes interact with, or are substrates of, MDR1 (Ambudkar et al., 1999), BCRP (Doyle et al., 1998; Litman et al., 2000), and the different MRPs (Borst et al., 2000). Furthermore, there is a partial substrate overlap among MDR1, BCRP, and the MRPs. A complex picture is emerging in which the pharmacokinetics and pharmacodynamics of a drug can be influenced not only by more than one efflux protein but also by functional polymorphism in those proteins (Hoffmeyer et al., 2000). However, this complexity will only be apparent if efflux proteins are expressed at significant levels in human tissues. A number of publications have presented human expression data for these efflux proteins, but only scattered

ABBREVIATIONS: ABC, ATP-binding cassette; BCRP, breast cancer resistance protein; LRP, lung resistance-related protein; PCR, polymerase chain reaction; RT-PCR, reverse transcription-PCR.

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information is available regarding their expression in the human small intestine, a rate-limiting barrier to oral drug absorption (e.g., Kool et al., 1997; Doyle et al., 1998; Fromm et al., 2000; Maliepaard et al., 2001). A systematic investigation of a wider selection of efflux proteins in the human small intestine is therefore warranted.

Studies on the role of efflux mechanisms in the absorption of drugs are generally performed in cell culture models such as the human intestinal epithelial Caco-2 cell line (Braun et al., 2000). However, as is the case for human tissues, only limited and mainly qualitative data on efflux protein expression in this cell line are available (e.g., Gutmann et al., 1999; Hirohashi et al., 2000). A lack of awareness of the expression of multiple efflux systems in the cell lines, as well as in vivo, may potentially result in the erroneous classification of drugs as being, for example, solely MDR1 substrates, since the drugs could also be transported by other efflux proteins with overlapping substrate specificity (e.g., BCRP). An investigation into the extent of expression of the various efflux proteins at the mRNA level in cell culture models would be a first step in resolving this problem. In addition, a quantitative comparison in this respect between the most important absorption site for orally administered drugs, the human jejunum, and the Caco-2 cell line will provide insight into the usefulness of this cell line as a model for oral drug absorption.

We therefore investigated the mRNA expression levels of 10 efflux proteins of the ABC transporter superfamily in both the human jejunum and well differentiated Caco-2 cell monolayers grown on permeable support. In addition, we investigated the mRNA expression levels of lung resistance-related protein (LRP) and CYP3A4. Since jejunal biopsies from 13 healthy human subjects were used, it was also possible to perform a preliminary investigation into the interindividual variability of all mRNA levels.

**Materials and Methods**

**Tissue Samples and Cell Cultures.** Human jejunal mucosa biopsies were obtained using a Watson capsule from 13 healthy volunteers (on no medication) ages 21 to 52 years (four female and nine male). Biopsies and all subsequent procedures were carried out with consent from the ethical review board of Huddinge University Hospital.

Caco-2 cells, a colorectal carcinoma cell line, were obtained from American Type Culture Collection (ATCC, Manassas, VA) and cultured at passage number 99 on Transwell polycarbonate filters (Costar, Cambridge, MA) for 21 days, as previously described (Arturson et al., 1996). For RNA isolation, filter-grown cells were harvested using ice-cold phosphate-buffered saline and a cell scraper. Cells were kept on ice during the scraping procedure and subsequently recovered by centrifugation.

Tissue and cell samples were frozen in liquid nitrogen and then stored at −80°C until required for RNA preparation.

**RNA Extraction, Quantitation and Quality Check.** Total RNA was isolated using the RNeasy mini kit (QIAGEN GmbH, Hilden, Germany) following the instructions provided by the manufacturer, with an additional on-column DNase treatment step (QIAGEN). Cells and tissues were homogenized using 3 sequential pulses (of homogenization) of 20 s each using a Heidolph DIAX 900 tissue homogenizer equipped with a 6G tool (Heidolph Instruments, Cinnaminson, NJ). RNA was quantified using the Ribogreen reagent from Molecular Probes (Eugene, OR), and RNA integrity was checked by assessing the sharpness of ribosomal RNA bands on a native 1% agarose gel using a 1× TBE running buffer (0.09 M Tris-borate, 0.002 M EDTA, pH 7.8). The gel was run at 2.5 V/cm for 40 min.

**Standards for Quantitative Real-Time PCR (TaqMan) Analysis.** Two types of DNA standard were used for quantitative estimates of transcript abundances: plasmid cDNA clones and genespecific PCR products.

cDNA plasmid clones corresponding to the following transcripts were generously provided by the cited authors: MDR1 (Chen et al., 1997), MRP1 (Cole et al., 1992), MRP2 (GenBank accession number U49248, direct submission by Kool et al., 1997), MRP3 (Kool et al., 1999), MRP5 (McAleer et al., 1999), BCRP (Doyle et al., 1998), and LRP (Scheffer et al., 1995). A plasmid harboring the MDR3 cDNA was purchased from ATCC. Plasmids were propagated using standard procedures and isolated using the QIAfilter Plasmid midi kit (QIAGEN).

cDNA fragments (see Table 1 and 2) corresponding to MRP4 (accession number AF071202), MRP6 (accession number AF076622), villin (accession number X12801), and CYP3A4 (accession number M14096) that covered the TaqMan primer/probe area were PCR-amplified from reverse-transcribed Caco-2 cell RNA using the QIAGEN OneStep RT-PCR kit. A PCR product corresponding to ABCG5 (accession number AC002486) was generated from genomic Caco-2 DNA using the HotStarTaq DNA polymerase kit (QIAGEN). All PCR products were purified using the GenElute PCR DNA purification kit (Sigma, St. Louis, MO).

All of the DNA standards were quantified using the PicoGreen reagent (Molecular Probes). Each standard curve was generated

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**TABLE 1**

Gene-specific PCR primers

<table>
<thead>
<tr>
<th>Gene&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Forward Primer&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Reverse Primer&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Base Pair&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR1 (ABCB1)</td>
<td>5'-CTCATGTGTTTGTCTAGATCGT</td>
<td>5'-GCTCTTCGTGTCGGCTGTTT</td>
<td>1873–2160</td>
</tr>
<tr>
<td>MDR3 (ABCB4)</td>
<td>5'-TGCAGCAAGAGGACCCAC</td>
<td>5'-TGGCTTGGGCAATCTGTTT</td>
<td>1588–2193</td>
</tr>
<tr>
<td>ABCB5</td>
<td>5'-GACATGCGTGGTTTATGATTGCT</td>
<td>5'-GAATCAGGACCCTGGAGACA</td>
<td>2639–3305</td>
</tr>
<tr>
<td>MRP1 (ABCC1)</td>
<td>5'-CATGAAAGGCCTAGGCACCTCT</td>
<td>5'-AGGCTTCCGTGGAACAGACAA</td>
<td>1858–2845</td>
</tr>
<tr>
<td>MRP2 (ABCC2)</td>
<td>5'-GACTATGGGCTGATACCTAGCT</td>
<td>5'-CCGGAGCTTTCAGAATTGAC</td>
<td>2687–3176</td>
</tr>
<tr>
<td>MRP3 (ABCC3)</td>
<td>5'-GGCTCTGCTGATAGTGGAGACA</td>
<td>5'-AAATGCGTTCTTCTCCCTCTCCT</td>
<td>2639–2867</td>
</tr>
<tr>
<td>MRP4 (ABCC4)</td>
<td>5'-AGTGACAACACCTCCAGTCCA</td>
<td>5'-CCGGAGCTTTCGAAAGGAGGAG</td>
<td>2026–2543</td>
</tr>
<tr>
<td>MRP5 (ABCC5)</td>
<td>5'-CATCAGCGTCTAGAAGGCAAT</td>
<td>5'-GAGCTTCCGAGGAGAGA</td>
<td>2686–3305</td>
</tr>
<tr>
<td>MRP6 (ABCC6)</td>
<td>5'-TCCAGAGCCCAAGACAGGAGG</td>
<td>5'-CCAGGCTTACGAGGAACAA</td>
<td>2704–3300</td>
</tr>
<tr>
<td>BCRP (ABCC2)</td>
<td>5'-CTTGACGCTGTTCTGATCCGAGGAGTTT</td>
<td>5'-TGAGTCTGGTGGAAGGAG</td>
<td>1563–2024</td>
</tr>
<tr>
<td>LRP (MVP)</td>
<td>5'-CAATGGCAGCTATGGGAGGAGGAG</td>
<td>5'-TGGAGGATTGTGTTGATCTT</td>
<td>1734–2112</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>5'-GAAGGTGCTGCTGGAAGTGAGACACC</td>
<td>5'-TCAGTCTGCTGCTGACAT</td>
<td>780–1004</td>
</tr>
<tr>
<td>Villin (VIL1)</td>
<td>5'-AAACGCAAGTGCAAGGAGGAAGGAG</td>
<td>5'-ATGAGTCTGCTGCTGACAT</td>
<td>928–1163</td>
</tr>
</tbody>
</table>

<sup>a</sup>Name within parentheses or single name corresponds to the Homo sapiens Official Gene Symbol, according to the HUGO Gene Nomenclature Committee (http://www.gene.ucl.ac.uk/nomenclature/).

<sup>b</sup>An additional ACTGGCTC sequence was incorporated at the 5'-end of both the forward and the reverse primer.

<sup>c</sup>Amplicon length is given in base pairs.

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<sup>c</sup>Amplicon length is given in base pairs.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR1</td>
<td>5′-TCAGGAAGCCAGATCCAGTCA-3′</td>
<td>5′-AGTGGCAGCCTTTTCATCATTTAGTTCAAATTCTT-3′</td>
</tr>
<tr>
<td>MDR2</td>
<td>5′-AGACATCGGCTGGTTTGATAGCT-3′</td>
<td>5′-TGACATCGGTGAACTTAACACTCGCATGAGTTTCCAGCCCTTCACCA-3′</td>
</tr>
<tr>
<td>MRP1</td>
<td>5′-GAAGGCCATCGGACTCTTCA-3′</td>
<td>5′-CTCCTTCCTCAGCATCTTCCTTTTCATGTGCAGCGCGGACACATGGT-3′</td>
</tr>
<tr>
<td>MRP2</td>
<td>5′-CACACGGATCTGACAGACAATGAC-3′</td>
<td>5′-CCAGTCACCTATGTGGTCCAGAAGCAGTTCACAGGGCACTCAGCTGTCTC-3′</td>
</tr>
<tr>
<td>MRP3</td>
<td>5′-MRP6</td>
<td>5′-AGACACGGTTGACGTGGACAT-3′</td>
</tr>
<tr>
<td>BCRP</td>
<td>5′-GGCCTCTGTCACTTTCGATGAT-3′</td>
<td>5′-TTCCATAAGAACTCCCGCATCATTTCCTTCCGAGGTCTCAAAGCA-3′</td>
</tr>
<tr>
<td>LRP</td>
<td>5′-AGAAAGTCGCCTCGAAGATACAC-3′</td>
<td>5′-TCTGGAGCTCGTGGCCCAATCAATTATCTGCAGTTTCTGCTGGACATC-3′</td>
</tr>
<tr>
<td>Villin</td>
<td>5′-Villin</td>
<td>5′-Villin</td>
</tr>
</tbody>
</table>

* Gene within parenthesis or single prime corresponds to the Gene symbols given by the International Nomenclature Committee for Human Genes. Official Gene Symbol, according to the HUGO Gene Nomenclature Committee (http://www.gene.ucl.ac.uk/nomenclature/).

**Table 2**

Gene-specific primers and probes for TaqMan analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR1</td>
<td>5′-TCAGGAAGCCAGATCCAGTCA-3′</td>
<td>5′-AGTGGCAGCCTTTTCATCATTTAGTTCAAATTCTT-3′</td>
</tr>
<tr>
<td>MDR2</td>
<td>5′-AGACATCGGCTGGTTTGATAGCT-3′</td>
<td>5′-TGACATCGGTGAACTTAACACTCGCATGAGTTTCCAGCCCTTCACCA-3′</td>
</tr>
<tr>
<td>MRP1</td>
<td>5′-GAAGGCCATCGGACTCTTCA-3′</td>
<td>5′-CTCCTTCCTCAGCATCTTCCTTTTCATGTGCAGCGCGGACACATGGT-3′</td>
</tr>
<tr>
<td>MRP2</td>
<td>5′-CACACGGATCTGACAGACAATGAC-3′</td>
<td>5′-CCAGTCACCTATGTGGTCCAGAAGCAGTTCACAGGGCACTCAGCTGTCTC-3′</td>
</tr>
<tr>
<td>MRP3</td>
<td>5′-MRP6</td>
<td>5′-AGACACGGTTGACGTGGACAT-3′</td>
</tr>
<tr>
<td>BCRP</td>
<td>5′-GGCCTCTGTCACTTTCGATGAT-3′</td>
<td>5′-TTCCATAAGAACTCCCGCATCATTTCCTTCCGAGGTCTCAAAGCA-3′</td>
</tr>
<tr>
<td>LRP</td>
<td>5′-AGAAAGTCGCCTCGAAGATACAC-3′</td>
<td>5′-TCTGGAGCTCGTGGCCCAATCAATTATCTGCAGTTTCTGCTGGACATC-3′</td>
</tr>
<tr>
<td>Villin</td>
<td>5′-Villin</td>
<td>5′-Villin</td>
</tr>
</tbody>
</table>

* Area amplified refers to sequence deposited at GenBank with accession numbers as follows: MDR1 (M23234), MRP2 (AF071202), MRP3 (AF071202), MRP6 (AF071202).

from a number of copies that ranged from $10^3$ to $10^7$ copies (single strands of DNA) per 25-μl TaqMan reaction.

**Gene-Specific PCR.** Gene-specific PCR-primers (Table 1) were developed by performing a multiple sequence alignment within each of the different gene subfamilies using the Clustal W program, available through the Biology WorkBench (http://workbench.sdsc.edu/). Primers were constructed using the PRIMER3 software, also available through the Biology WorkBench and selected based on the multiple sequence alignments. All of the PCR primers were manufactured by Interactiva (Ulm, Germany).

Gene-specific RT-PCR was carried out on total RNA from filter-grown Caco-2 cells using a master mix based on the QIAGEN One-Step RT-PCR kit, supplemented with 12.5 U of SUPERase-In (Ambion, Austin, TX) and 200 ng of total RNA per 25-μl reaction. For each RT-PCR reaction, appropriate primers were added to give a final concentration of 600 nM each primer. Following an initial gene-specific reverse transcription step, each of the reactions went through an initial PCR cycle at an annealing temperature of 56°C, after which the different reactions went through an additional 24 to 34 cycles, depending on the prevalence of the transcript in question, at an elevated annealing temperature (62°C). Thermal cycling was conducted using an UNO II thermocycler from Biometra (Göttingen, Germany).

The RNA samples were not contaminated by genomic DNA, as was shown by omitting the reverse transcription step from otherwise identical reactions. Furthermore, no cross-reactivity was observed during PCR amplification when using the different plasmids described above [see Standards for Quantitative Real-Time PCR (TaqMan) Analysis] as controls.

We observed expression from all genes except ABCB5 using total RNA from filter-grown Caco-2 cells (data not shown). Furthermore, all PCR fragments except MDR3 migrated as a single product of the expected size on an agarose gel. PCR products corresponding to the MDR3 transcript migrated as a double band, both having approximately the same intensity, one fragment of the expected size and one slightly larger. Since no expression of ABCB5 was observed, the ABCB5 PCR product was amplified from genomic DNA and used as a DNA standard for the TaqMan analysis.

**Real-Time Quantitative PCR (TaqMan) Analysis of Transcript Abundance.** Suitable gene-specific primer/probe combinations (Table 2) were selected, based on multiple sequence alignments described above (see Gene-Specific PCR), using the Primer Express software (Applied Biosystems, Foster City, CA). TaqMan probes were obtained from Applied Biosystems.

The TaqMan analysis was carried out using 1.5 μg of total RNA, which was reverse transcribed in a 100-μl reaction using random hexamers and the TaqMan reverse transcription reagent (Applied Biosystems). From this, 25 ng of reverse-transcribed RNA was used for each 25-μl TaqMan reaction. The different TaqMan assays were performed using a master mix based on the TaqMan universal PCR master mix (Applied Biosystems). This mix contained the appropriate primer/probe combination. Each primer and probe was used at final concentrations of 300 and 200 nM, respectively. The appropriate reverse-transcribed RNA was added to aliquots of this master mix. These aliquots were subsequently split into duplicate samples (unless otherwise indicated) and run on the ABI PRISM 7700 sequence detection system (Applied Biosystems).

TaqMan readings were normalized using the number of villin transcripts determined for each biopsy. This was accomplished by using one of the biopsies as a standard and calculating the ratio between the numbers of villin transcripts determined for the standard and for the biopsy in question. For each biopsy, this ratio was subsequently multiplied with the number of transcripts determined for the gene in question (e.g., MDR1<sub>biopsy</sub> · villin<sub>biopsy</sub>/villin<sub>standard</sub> · villin<sub>biopsy</sub>). Villin was chosen since, in the intestine, villin and at least MDR1, MRP1, MRP2, and CYP3A4 are exclusively expressed in the epithelial cells (Thiebaut et al., 1987; West et al., 1988; Kolars et al., 1994; Peng et al., 1999; Fromm et al., 2000). Furthermore, villin has
higher levels than the Transcripts coding for BCRP and MRP2 were expressed at the hitherto most intensively studied efflux protein, MDR1. Proteins were comparable with and even higher than that of 3 and Fig. 1). Indeed, the transcript level for six of the efflux transcripts were only detected at a very low level. In total, all of the efflux protein genes previously been established as a useful measure to control for variation in the enterocyte content of biopsies (Lown et al., 1997).

Results

Quantitative Analysis of Efflux Protein Transcript Levels in Human Jejunum. All of the efflux protein genes except ABCB5 were expressed in the human jejunum (Table 3 and Fig. 1). Indeed, the transcript level for six of the efflux proteins were comparable with and even higher than that of the hitherto most intensively studied efflux protein, MDR1. Transcripts coding for BCRP and MRP2 were expressed at higher levels than the MDR1 transcripts, whereas MDR3 transcripts were only detected at a very low level. In total, transcript levels spanned a range of three logs, with the maximum and minimum values among the subjects.

Table 3

<table>
<thead>
<tr>
<th>Transcripts</th>
<th>Jejunum Mean ± S.D.</th>
<th>Minimum–Maximum</th>
<th>Caco-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR1/10⁵</td>
<td>7.9 ± 1.4</td>
<td>5.3–10.3</td>
<td>11.0</td>
</tr>
<tr>
<td>MDR3/10³</td>
<td>5.2 ± 3.0</td>
<td>2.5–12.9</td>
<td>1.5</td>
</tr>
<tr>
<td>MRP1/10⁷</td>
<td>1.8 ± 0.5</td>
<td>1.1–2.5</td>
<td>0.8</td>
</tr>
<tr>
<td>MRP2/10⁷</td>
<td>2.4 ± 0.6</td>
<td>1.7–3.3</td>
<td>4.9</td>
</tr>
<tr>
<td>MRP3/10⁷</td>
<td>4.6 ± 1.3</td>
<td>2.5–6.9</td>
<td>22.1</td>
</tr>
<tr>
<td>MRP4/10⁰</td>
<td>6.5 ± 1.3</td>
<td>5.1–9.4</td>
<td>34.6</td>
</tr>
<tr>
<td>MRP5/10⁰</td>
<td>2.1 ± 0.5</td>
<td>1.2–3.0</td>
<td>1.3</td>
</tr>
<tr>
<td>MRP6/10⁰</td>
<td>2.5 ± 0.7</td>
<td>1.4–3.7</td>
<td>6.3</td>
</tr>
<tr>
<td>BCRP/10⁹</td>
<td>2.7 ± 0.6</td>
<td>2.1–3.7</td>
<td>0.03</td>
</tr>
<tr>
<td>LRP/10⁶</td>
<td>2.7 ± 0.8</td>
<td>1.8–4.5</td>
<td>0.2</td>
</tr>
<tr>
<td>CYP3A4/10⁷</td>
<td>1.4 ± 0.3</td>
<td>0.8–1.8</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

a The /10ⁿ in the Transcript column is the multiplier for the mean ± S.D. column. b Results are based on 13 jejunal biopsies and presented as mean ± S.D. The maximum and minimum values for each transcript are also given.

The interindividual variation in transcript abundance was low for all investigated genes except MDR3, with a difference between the highest and lowest value of around a factor of 2 (range 1.7–2.7, see Table 3). The MDR3 transcripts, which were present at a considerably lower level than those of the other transcripts, varied by a factor of 5.3 between the highest and lowest values. Nevertheless, in many of the jejunal biopsies, MDR3 exhibited quite similar expression levels.

Statistical Analysis of Interindividual Variation in MDR1 Expression. Since an average difference of two in duodenal MDR1 expression seemed sufficient for affecting digoxin plasma levels (Hoffmeyer et al., 2000), it was of interest to determine the extent to which variations in transcript copy numbers can be attributed to interindividual variations and how much are experimental errors inherent in the TaqMan assay. For this purpose, we performed three independent TaqMan assays using the same reverse-transcribed total RNA to investigate the MDR1 transcript copy numbers of the different jejunal biopsies. The three data sets did not result in entirely consistent rankings of the individual biopsies with respect to MDR1 expression (Fig. 2), which shows that at least some of the variation is due to experimental errors. Nevertheless, one-way analysis of variance and Tukey’s multiple comparison test indicated that 66% of the observed variation could be attributed to the variation between individuals.

The difference between duplicate TaqMan assays was generally less than approximately 10% of each duplicate’s mean for all genes and samples analyzed, and for almost all of the duplicates this difference was less than 30%. Independently performed MDR1 TaqMan assays resulted in standard deviations of more than 10% of the mean (but below 20% for 12 of the 13 biopsies). For many of the tissue samples, transcript copy numbers of the different genes differed by less than this; hence, it is not possible to rank the different biopsies with respect to gene expression data reliably. This means that we cannot analyze our data set for concerted changes in gene expression. Nevertheless, even though small differences between samples are not easily resolved using this assay, TaqMan is still suitable for rapidly obtaining a quantitative estimate of transcript levels, especially when analyzing gene expression data for many genes and numerous tissue samples of limited supply.

Evaluation of the Caco-2 Cell versus Human Jejunum. In general, the extent of the expression of the genes responsible for the efflux systems in filter-grown Caco-2 cell monolayers was in good agreement with that in the human jejunum (Table 3 and Fig. 3). A comparison of villin-normal-
Levels in Human Jejunum.

To our knowledge, this is the first investigation that describes the quantitative expression of 10 efflux protein transcripts (both apically and basolaterally localized ones) in normal human jejunum, the primary site of absorption of orally administered drugs. Surprisingly, six of ten of the efflux protein transcripts were expressed at levels comparable with, and in the case of BCRP and MRP2 even higher than, the MDR1 transcript. MDR1 is the most intensively investigated efflux protein. Since there is clear evidence that many clinically important drugs are substrates for MDR1 and that polymorphisms in this gene affect the pharmacokinetics of digoxin (Hoffmeyer et al., 2000), a drug with a very narrow therapeutic window, our results suggest that increased attention should now be given to several other abundantly expressed efflux systems in the small intestine.

Previous reports describe qualitative expression patterns for some of the efflux proteins investigated here. However, these communications only provide a sketchy picture of intestinal efflux protein expression (e.g., Kool et al., 1997; Doyle et al., 1998; Fromm et al., 2000; Maliepaard et al., 2001). Furthermore, almost none of these studies were concerned with jejunum, which is the most important drug absorption site of the human small intestine. The exceptions are MDR1 and MRP2 for which expression was demonstrated in human and rat jejunum, respectively (Thiebaut et al., 1987; Gotoh et al., 2000; Mottino et al., 1999).

The MDR1, MDR3, MRP2, and BCRP proteins are all located at the apical part of the plasma membrane of polarized cells such as epithelia (Thiebaut et al., 1987; van Helvoort et al., 1996; Fromm et al., 2000; Jonker et al., 2000). If the comparatively high mRNA expressions of BCRP and MRP2 are reflected in high levels of protein, it will most certainly have bearing on the interpretation of drug absorption of orally administered drugs that are substrates for these proteins. Indeed, intestinal expression of BCRP in mice results in a lower bioavailability of orally administered topotecan (Jonker et al., 2000). Also, in vivo data obtained using MRP2-defective rats indicate that MRP2 may be involved in intestinal drug efflux (Gotoh et al., 2000).

The substrate specificities of the efflux proteins partly overlap, and a complex picture is emerging in which several drug transport and efflux routes may be active in parallel (Chen et al., 2000; Litman et al., 2000). For example, in addition to topotecan, BCRP confers resistance to, or reduced accumulation of, important drugs such as mitoxantrone, doxorubicin, daunorubicin, prazosin, and bisantrene (Doyle et al., 1998; Litman et al., 2000), and many of the BCRP substrates are also substrates of MDR1 (Ambudkar et al., 1999). However, at least mitoxantrone and topotecan might be better substrates for BCRP (Litman et al., 2000). Furthermore, although both topotecan and prazosin are administered orally, BCRP is only a major determinant of oral bioavailability of topotecan (Jonker et al., 2000). Prazosin, on the other hand, is well absorbed through the intestine (Hoffman and Lefkowitz, 1996), which indicates that active efflux (through either BCRP or MDR1) is not a major determinant of the bioavailability of this compound.

The MRP1, MRP3, MRP5, and MRP6 proteins seem to be basolaterally localized (Roelofsen et al., 1997; Kool et al., 1999; Madon et al., 2000; Wijnholds et al.), and we found relatively high expression levels of these transcripts in human normal jejunum, particularly for the MRP3 transcript. However, we cannot find any published evidence on the role of these efflux proteins in drug absorption. It therefore remains to be elucidated whether or not the intestinal expression of these basolaterally localized efflux proteins can influence the absorption of orally administered drugs.

**Discussion**

**Quantitative Analysis of Efflux Protein Transcript Levels in Human Jejunum.** To our knowledge, this is the first investigation that describes the quantitative expression of 10 efflux protein transcripts (both apically and basolaterally localized ones) in normal human jejunum, the primary site of absorption of orally administered drugs. Surprisingly, six of ten of the efflux protein transcripts were expressed at levels comparable with, and in the case of BCRP and MRP2...
The LRP protein, which constitutes a part of the multicomponent vault complexes, is frequently overexpressed in multidrug-resistant cancer cells and may mediate drug resistance via a transport process (Scheffer et al., 1995). We included the LRP protein in this study for this reason. However, the role of LRP in drug resistance is far from clear, and it is possible that LRP is only linked to the up-regulation of other processes involved in multidrug resistance. The LRP gene is located close to the chromosomal region of MRP (MRP1 and MRP6) and a gene for a protein kinase C that is involved in the up-regulation of MDR1 activity (Scheffer et al., 1995). Clearly, further studies on the functional role of LRP are needed before the significance of the present findings can be established.

**Interindividual Variation in the Expression of Multidrug Resistance-Related Genes in Human Jejunum.**

The interindividual variation in transcript abundance was low for all investigated genes except MDR3, with a difference between the highest and lowest value of around a factor of 2. The higher variability of MDR3 expression might be caused by the existence of alternative transcripts, as is observed in the RNA from filter-grown Caco-2 cells (see Gene-specific PCR under Materials and Methods). This was, however, not investigated.

We conclude that at least some of the observed variation is due to interindividual variation in transcript abundance but the present data set does not allow a reliable ranking of the different individuals (see Statistical Analysis of Interindividual Variation in MDR1 Expression). Among these 13 individuals, no extreme phenotype can be found for any of the genes investigated. Interindividual variations in expression of up to 10-fold (Lown et al., 1995, 1997) and up to 65-fold (Hoffmeyer et al., 2000) have been reported for MDR1, and up to 30-fold for CYP3A4 (Wacher et al., 1998). Still, interindividual variation in CYP3A4 expression for most of the subjects was considerably more modest (Lown et al., 1997; Fontana et al., 1999), as is also reported in this communication. This is also the case for MDR1. Nevertheless, the homozygous 3435T allele is associated with a 2-fold reduced MDR1 expression and an increased digoxin plasma concentration following oral administration (Hoffmeyer et al., 2000).

A parallel investigation of interindividual variability in the expression of MDR1 and CYP3A4 proteins, and possibly other proteins (e.g., BCRP), is warranted because of the overlap in substrate use between these proteins, and because metabolism by intestinal CYP3A4 is a major determinant of the bioavailability of orally administered drugs (Wacher et al., 1998). Only in this way will the real impact of MDR1 to interpatient variability in oral drug bioavailability be established.

**Evaluation of the Caco-2 Cell versus Human Jejunum.**

A comparison of villin-normalized data indicated that the expression of efflux protein genes in Caco-2 cells generally differed by a factor of less than 2.5 from that in the jejunum, a result that is comparable with the interindividual variations observed in this and other studies (vide supra). The exception was BCRP.

However, functional studies have indicated a larger efflux of MDR1 substrates in Caco-2 cells than in the human intestine, which could, thus, be interpreted as an overexpression of MDR1 in Caco-2 cells (Yee, 1997; Gres et al., 1998). Our results challenge this interpretation and are further corroborated by a recent study of MDR1-mediated efflux of digoxin and vinblastin across Caco-2 cell monolayers and human and rat intestinal segments (Stephens et al., 2001). Stephens et al. (2001) suggests that it may be possible to predict small intestinal drug efflux quantitatively using Caco-2 cells. However, the variable expression of MDR1 reported for various Caco-2 clones and culturing conditions (Delie and Rubas, 1997) suggests that transcript levels in other Caco-2 clones may not correlate as well with human jejunum as the results presented here. Indeed, preliminary results indicate some variability in the MDR1 expression level, both within and between Caco-2 clones (J. Taipalensuu, unpublished observation). Nevertheless, our data seems to fit rather well with the Northern blot analysis of MRP2, MRP3, and MRP5 expression in Caco-2 cells presented by Hirohashi et al. (2000). In the case of MRP1, however, there seems to be a discrepancy between data. This discrepancy might simply be a result of the different methods used (TaqMan versus Northern blot analysis) or, more likely, caused by Caco-2 clone heterogeneity.

**Conclusions**

This study indicates that several efflux proteins are abundantly expressed in the healthy human jejunum. In particular, BCRP and MRP2 are more extensively expressed than MDR1, and the roles of these two drug efflux proteins as barriers to intestinal drug absorption are most likely more important than has previously been suspected. Since the substrate specificities of BCRP and MRP2 partly overlap with that of MDR1, it is clear that all of these efflux systems will contribute to a greater or lesser extent to the absorption of such substrates. Furthermore, the results indicate that the Caco-2 cells capture the expression of investigated drug efflux systems, with the exception of BCRP, of the healthy human jejunum. It may therefore be beneficial to complement a Caco-2-based drug efflux screening model with a system that expresses BCRP to cover all important drug efflux systems in the human jejunum. The findings of this study need to be verified at the protein and functional levels.

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