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Gene expression of CYP3A4, ABC-transporters (MDR1 and MRP1–MRP5) and hPXR in three different human colon carcinoma cell lines

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Abstract

Colon carcinoma cell lines are used widely as screening models for intestinal absorption of drugs. However, the expression of important transport systems and of metabolic enzymes is not completely characterized yet. The expression and inducibility of multidrug resistance gene 1 (MDR1) and cytochrome P450 isoform 3A4 (CYP3A4) was investigated in Caco-2 parental, Caco-2 TC-7 (TC-7) and LS180 cell lines. In the same three cell lines, we investigated the expression of isoforms of the multidrug resistance associated protein family (MRP1–MRP5) and the human pregnane X receptor (hPXR), which may be important for MDR1 and CYP3A4 induction. Cells were treated with rifampicin or 1α,25-dihydroxycholecalciferol (1,25(OH)$_2$D$_3$) for 72 h and the total RNA was extracted. Afterwards reverse transcription real-time polymerase chain reaction (TaqMan) assay was performed to determine the mRNA expression level. We have shown that in LS180 cells, MDR1 and CYP3A4 were inducible with both inducers. In Caco-2 parental and TC-7 cells, CYP3A4 was only inducible with 1,25(OH)$_2$D$_3$. Furthermore, differences were shown in gene expression of several transport proteins (MDR1 and MRP1–MRP5) and CYP3A4 in different human colon carcinoma derived cell lines. hPXR mRNA was expressed in all three cell lines but the amount of mRNA detected was significantly higher in LS180 cells than in Caco-2 and TC-7 cells. We concluded that LS180 cells were a suitable model to study MDR1 and CYP3A4 induction, but for drug transport studies Caco-2 parental and TC-7 cells would be preferred as the more physiological model.

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Introduction

The mucosa of the small intestine is known to be an important determinant of drug absorption and metabolism (Watkins 1997). The adenosine triphosphate (ATP)-binding cassette transmembrane transporter P-glycoprotein (P-gp) and the drug metabolizing enzyme cytochrome P450 3A4 (CYP3A4) are recognized as potentially important barriers to drug absorption (Wrighton et al 2000). Inhibition and induction of prehepatic and hepatic metabolism and also of P-gp have been shown to determine the systemic availability of orally administered drugs. P-gp, the gene product of the multidrug resistance protein 1 (MDR1) gene, is an efflux transporter with wide substrate specificity. The protein is expressed on the brush-border membrane of enterocytes where it extrudes its substrates back into the gut lumen (Watkins 1997; Ambudkar et al 1999). Since the tissue distribution and the substrate specificity of CYP3A4 and P-gp overlap each other, it is assumed that the action of both proteins results in a reduced bioavailability of their substrates after oral administration (Wacher et al 1995; Kim et al 1999; Zhang & Benet 2001). Interestingly, CYP3A4 and MDR1 can be induced by many of the same compounds (Kolars et al 1992) such as rifampicin (Fromm et al 1996; Greiner et al 1999; Kyrklund et al 2000) and St John's Wort extract (Durr et al 2000). Thus CYP3A4 and P-gp induction could be a reason for drug–drug interactions (Krishna & Klotz 1994; Fromm et al 1996; Greiner et al 1999; Kosel & Aweeka 2000).

Only recently has the molecular mechanism of CYP3A4 and P-gp induction been elucidated. Human pregnane X receptor (hPXR), an orphan nuclear receptor, was

described as a key regulator of CYP3A4 and P-gp transcription and other ABC transporters and cytochrome isoforms. After hPXR activation by specific ligands such as dexamethasone-t-butylacetate, RU486, rifampicin, clotrimazole and others, hPXR builds heterodimers with retinoic X receptor (RXR), another nuclear receptor, and then interacts with a specific DNA sequence called hormone-binding element. Many of the compounds that induce CYP3A4 and/or P-gp expression bind directly to hPXR (Lehmann et al 1998; Geick et al 2001; Synold et al 2001).

Multidrug resistance related proteins (MRPs) are integral membrane glycoproteins and function as organic anion transporters, which transport a wide range of drugs. MRP1, MRP4 and MRP5 mRNAs are widely distributed in the body where as MRP2, MRP3 and MRP6 appear to function predominantly in liver, kidney and gut. MRP2 is located in apical and MRP1 and MRP3 in basolateral membranes of tissues (Borst et al 1999). As MRPs are widely expressed in the gut, their inhibition or induction could also contribute to an altered oral bioavailability. In contrast to P-gp, the potential role of MRPs for drug interaction in man has been evaluated only poorly.

In the process of drug development, several in-vitro models are used to evaluate oral drug absorption of new compounds. One of these widely used models is the human colon carcinoma derived Caco-2 cell line (Fogh et al 1977). It was reported to undergo spontaneous morphological and biochemical enterocytic differentiation. In culture, these cells develop a well-defined brush border on the apical surface as well as tight cellular junctions (Anderson et al 1989). Previously it has been shown that the Caco-2 cell line expresses P-gp, MRP1, MRP2, MRP3 and MRP5 (Gutmann et al 1999; Hirohashi et al 2000; Taipalensuu et al 2001). However, in contrast to the in-vivo situation of gut enterocytes, the CYP3A4 expression in Caco-2 cells is considerably low. Therefore, this cell line does not seem to be a suitable model to study absorption of intact drug molecules, when these drugs undergo CYP3A4 mediated metabolism. Several approaches were undertaken to overcome this disadvantage. Schmiedlin-Ren et al (1997) showed successful induction of CYP3A4 on mRNA and protein level in Caco-2 parental cells by treating them with 1α,25-dihydroxycholecalciferol (1,25$(OH)_2D_3$).

The Caco-2 TC-7 subclone is cloned from a late passage of the parental cell line by limited dilution technique (Chantret et al 1994). The morphological characteristics are similar to those of the parental Caco-2 cell line with respect to apical brush border, microvilli, tight junction and polarization of the cell line. The TC-7 subclone appeared to be more homogenous in terms of cell size and it showed an accelerated growth compared with the parental Caco-2 cells (Gres et al 1998). Engman et al (2001) showed that CYP3A4 mRNA and protein were induced in TC-7 cells after treatment with 1,25$(OH)_2D_3$.

LS180 is a human colon carcinoma derived cell line, which expresses microvilli. Schuetz et al (1996) showed that several drugs such as reserpine, rifampicin, phenobarbital and verapamil could increase P-gp and CYP3A4 mRNA and protein content in the LS180 cell line. Schmiedlin-Ren et al (2001) and Thummel et al (2001) showed that exposure to 1,25$(OH)_2D_3$ resulted in induction of CYP3A4 mRNA and CYP3A immunoreactive protein in the LS180 cell line.

With this background in mind, the aim of this study was to compare the inducibility of CYP3A4 and MDR1 and the expression of MRP1–MRP5 with quantitative real-time polymerase chain reaction (PCR) (TaqMan) analysis in three different cell lines (Caco-2 parental, Caco-2 TC-7 and LS 180). Rifampicin, a known in-vivo inducer of MDR1 and CYP3A4, and 1,25$(OH)_2D_3$, known to induce CYP3A4 mRNA and protein in Caco-2 parental cells, were used as substances to up regulate MDR1 and CYP3A4. The transcript level of hPXR, a regulator for MDR1 and CYP3A4 transcription, was assessed in each of the three cell lines.

Materials and Methods

Materials

All chemicals were of the highest quality available and were obtained from commercial sources.

Cell cultures

Caco-2 TC-7 cells were a kind gift from Dr M. Rousset (INSERM U505, Paris); they were used between passage 32 and 36. Caco-2 cells (used between passage 42 and 48) and the LS180 cell line (used between passage 36 and 40) were purchased from ATCC (Manassas, US). All three cell lines were cultured in Dulbecco's MEM with Glutamax-I, supplemented with 10% (v/v) foetal calf serum, 1% non-essential amino acids, 1% sodium pyruvate and 50 $\mu g\ mL^{-1}$ gentamicin. All cultures were maintained in a humidified 37°C incubator with a 5% carbon dioxide in air atmosphere. Former control experiments (data not shown) showed that 1,25$(OH)_2D_3$ induced CYP3A4 expression in Caco-2 cells up to 100-fold when grown on plastic culture dishes. The extent of induction was similar to that seen in Caco-2 cells grown on filters (Schmiedlin-Ren et al 1997). Therefore, all cells were seeded into six-well plastic culture dishes (9.2 cm^2/well). After the cells had reached confluence they were treated with the compound of interest for 72 h. The compounds were dissolved in dimethyl sulfoxide (DMSO). The following concentrations were applied: 0.25 μM 1,25$(OH)_2D_3$, 10 μM rifampicin (Schuetz et al 1996; Schmiedlin-Ren et al 1997). The final DMSO concentration did not exceed 1%. Up to this concentration, the solvent did not change significantly the expression level of any gene of interest compared with control without DMSO.

Real time polymerase chain reaction (TaqMan assay)

At the end of the culture period, the medium was removed and total RNA was extracted from the Caco-2 parental, TC-7 and LS180 cell lines using the RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA was quantified with a GeneQuant photometer (Pharmacia, Uppsala, Sweden).

Table 1 Sequences of PCR primers and specific probes for different targets.

Target sequence	Primer	Sequence (5′–3′)	GenBank accession number	Amplicon length
GAPDH	GAPDH forward	GGTGAAGGTCGGAGTCAACG		
	GAPDH probe	CGCCTGGTCACCAGGGCTGC	M17851	123
	GAPDH reverse	ACCATGTAGTTGAGGTCAATGAAGG		
MDR1	MDR1 forward	CTGTATTGTTTGCCACCACGA		
	MDR1 probe	AAGCTGTCAAGGAAGCCAATGCCTATGACTT	M14758	138
	MDR1 reverse	AGGGTGTCAAATTTATGAGGCAGT		
CYP3A4	CYP3A4 forward	TCTGGGATGAGAGCCATCACTA		
	CYP3A4 probe	TCCTTACTTATCTCTCTCCTCTGAGTCTTCCTTTCAGC	D11131	86
	CYP3A4 reverse	AGCCCAGCAAAGAGCAACAC		
MRP1	MRP1 forward	GGGCTGCGGAAAGTCGT		
	MRP1 probe	CCTCCACTTTGTCCATCTCAGCCAAGAG	NM_004996	80
	MRP1 reverse	AGCCCTTGATAGCCACGTG		
MRP2	MRP2 forward	ACTGTTGGCTTTGTTCTGTCCA		
	MRP2 probe	CTCAATATCACACAAACCCTGAACTGGCTG	NM_000392	99
	MRP2 reverse	CAACAGCCACAATGTTGGTCTCTA		
MRP3	MRP3 forward	GGTGGATGCCAACCAGAGAA		
	MRP3 probe	CCAACCGGTGGCTGAGCATCG	AF085690	85
	MRP3 reverse	GCAGTTCCCCACGAACTCC		
MRP4	MRP4 forward	ACCAGGAGGTGAAGCCCAAC		
	MRP4 probe	CGCTGCAGGACGCGAACATCTG	NM_005845	75
	MRP4 reverse	AGGGATTGAGCCACCAGAAGA		
MRP5	MRP5 forward	CTGCAGTACAGCTTGTTGTTAGTGC		
	MRP5 probe	CTGACGGAAATCGTGCGGTCTTGG	NM_005688	92
	MRP5 reverse	TCGGTAATTCAATGCCCAAGTC		
hPXR	hPXR forward	GGCCACTGGCTATCACTTCAA		
	hPXR probe	AGCCCTTGCATCCTTCACATGTCATGA	AF061056	71
	hPXR reverse	GTTTCATGGCCCTCCTGAAA		

Integrity of RNA after extraction was checked under these experimental conditions by ethidium bromide agarose gel electrophoresis. The purity of the RNA preparations was high as demonstrated by the 260 nm/280 nm ratio (range 1.8–2.0). After DNaseI digestion (Gibco, Life Technologies, Basel Switzerland) 1 μg total RNA was reversed transcribed by Superscript (Gibco, Life Technologies, Basel, Switzerland) according to the manufacturer's protocol using random hexamers as primers.

A total of 25 ng cDNA was used as a template for real-time quantitative PCR analysis, which was performed with the TaqMan assay using a Gene Amp 5700 Sequence Detector (Applied Biosystems, Rotkreuz, Switzerland), a combined thermocycler and fluorescence detector. A dual-labelled fluorogenic probe complementary to a sequence within each PCR product was added to the PCR reaction. The fluorescent dye at the 5′ end of the probe (6-carboxy-fluorescein) served as reporter, and its emission was quenched by the second fluorescent dye at the 3′ end of the probe (6-carboxy-tetramethyl-rhodamine). During elongation, the 5′ to 3′ exonuclease activity of the Taq DNA polymerase cleaved the probe, thus releasing the reporter from the quencher. Thus TaqMan detected increased fluorescence with every additional copy of the template.

Primers and probe were designed according to the guidelines of Applied Biosystems with help of the Primer Express 2.0 software (Applied Biosystems). Primers were custom synthesized by Invitrogen (Basel, Switzerland), probes by Eurogentec (Seraing, Belgium). The sequences of the primers and probes are listed in Table 1. The identity of the PCR products was verified by sequencing. The Complementary DNA (25 ng total RNA) was amplified in a 25 μL volume containing 12.5 μL Brilliant Quantitative PCR Core Reagent Kit components (Strateagene Amsterdam, The Netherlands), mixed after the manufacturer's protocol, 225 nM probe and 900 nM of each primer. Cycling conditions were 10 min 95°C initial denaturation and activation of AmpliTaq Gold DNA polymerase, followed by 40 cycles of 15 s 95°C denaturation, 1 min 60°C combined annealing and primer extension. A relative standard curve was generated by serial dilutions of cDNA. The cDNA with the highest concentration was set as 2× concentration. The dilution of the latter cDNA was expressed by the respective dilution value. Ct values of standards were plotted against the log of the respective dilution factors. Slope and y-intercept of the standard curve line were then calculated by linear regression and used to calculate the input amount for unknown samples for respective genes. To standardize the amount of sample cDNA added to the reaction the calculated amount of the gene of interest was divided by the calculated amount of the constitutively expressed glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene in the sample. These normalized amounts were then used to compare the relative amount of target in different samples.

The expression level of the endogenous control gene GAPDH did not alter under any of the different treatments.

Eventual contamination with genomic DNA was checked by TaqMan analysis of non-reverse transcribed RNA as negative control. No significant amplification was detected.

To generate the rank order of several ABC-transporters the differences in slopes of the regression lines were tested using residual and pooled sum of squares by the F-test (Zarr 1984). As the amplification efficiency (indicated as slope of the regression line) was not statistically different between all regression lines, the standard curve of the gene with the highest mRNA expression was used to quantify relative mRNA expression of all target genes.

Statistics

Data of groups were compared by analysis of variance. The level of significance was $P = 0.05$. If this analysis revealed significant differences, pair-wise comparison within groups was performed by the two-sided unpaired *t*-test. *P*-values were adjusted by Bonferroni's correction for multiple comparisons.

Results

Inducibility of MDR1 and CYP3A4

After having reached confluence, the cells were incubated with medium only, with rifampicin (10 μM) or with 1,25(OH)$_2$D$_3$ (0.25 μM) for 72 h. After RNA extraction and DNaseI digestion, TaqMan analysis was performed to determine the relative expression of mRNA from the different target genes.

The LS180 cell line showed a significant increase in MDR1 (Figure 1) and in CYP3A4 (Figure 1) mRNA level in the rifampicin and 1,25(OH)$_2$D$_3$ group compared with the LS180 control group. After rifampicin treatment the MDR1 mRNA level was increased approximately 7-fold, after 1,25(OH)$_2$D$_3$-treatment an induction of approximately 13-fold was measured. CYP3A4 mRNA expression was increased approximately 6-fold and more than 100-fold after rifampicin and 1,25(OH)$_2$D$_3$ treatment, respectively.

The MDR1 mRNA level in the Caco-2 parental and TC-7 cell lines was not significantly changed after either of the treatments. Rifampicin did not significantly influence the CYP3A4 mRNA level in the Caco-2 or TC-7 cell line. Treatment with 1,25(OH)$_2$D$_3$ induced CYP3A4 transcript level approximately 79-fold and 117-fold in Caco-2 and TC-7 cells, respectively.

Expression of CYP3A4, MDR1 and MRP transporters

To compare the CYP3A4, MDR1 and MRP1 to MRP5 mRNA expression level in the three different cell lines, the mRNA expression was normalized to that of untreated LS180 cells. Except for MRP1 where the mRNA level in all three cell lines was similar, the mRNA level of the MRP isoforms in LS180 was significantly lower compared with Caco-2 parental and TC-7 cells (data not shown). This difference was most pronounced for MRP2 where mRNA expression was 150-fold and 125-fold in Caco-2 and TC-7, respectively, compared with LS180. For MRP3, MRP4 and MRP5 the mRNA expression was 3.5–10-fold higher in Caco-2 and TC-7 compared with the LS180 control group. In Caco-2 parental cells the MDR1 mRNA level was approximately 140-fold, in TC-7 it was approximately

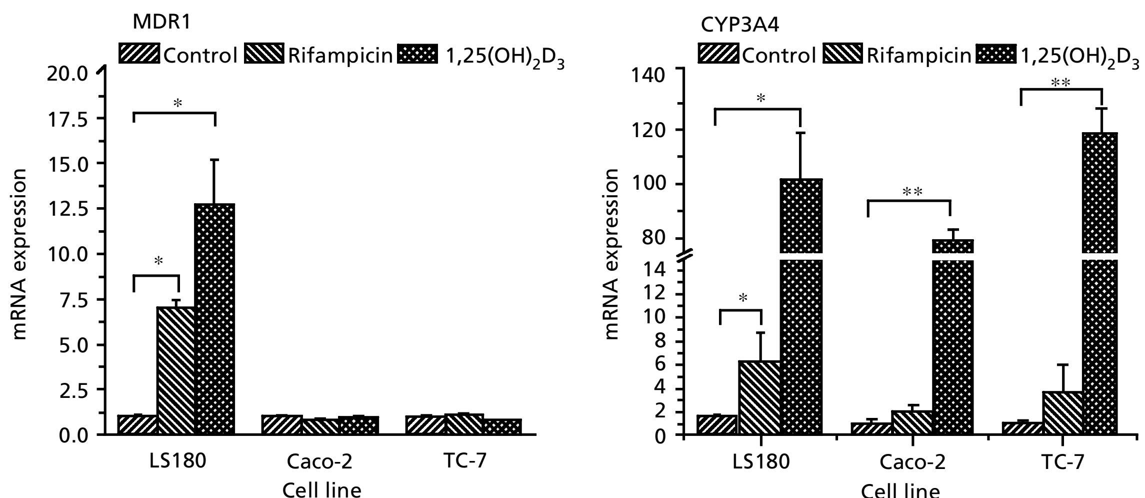

Figure 1 Relative mRNA expression of MDR1 and CYP3A4. Transcriptional expression of MDR1 and CYP3A4 was determined in LS180, Caco-2 and TC-7 cell lines by quantitative real-time PCR. Cells were treated with medium only or with either rifampicin (10 μM) or 1,25(OH)$_2$D$_3$(0.25 μM) for 72 h. mRNA expression of the cell line was relative to the respective control group. In LS180 cells MDR1 induction with rifampicin and 1,25(OH)$_2$D$_3$ was observed, whereas CYP3A4 gene expression was induced after 1,25(OH)$_2$D$_3$ treatment in all three cell lines. In LS180 cells rifampicin was able to induce CYP3A transcription. Data represent mean±s.e.m. of n = 3 experiments. $^*P < 0.05$, $^{**}P = < 0.001$.

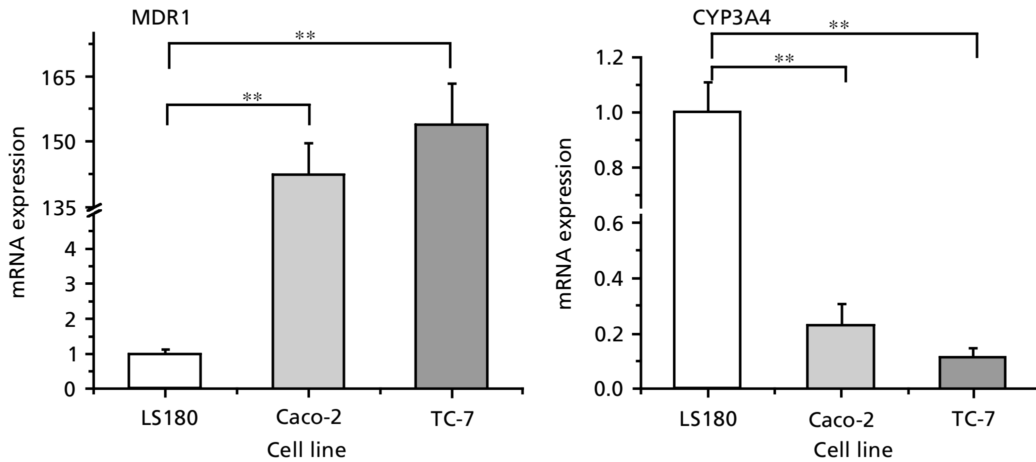

Figure 2 Relative mRNA expression of MDR1 and CYP3A4 in untreated LS180, Caco-2 parental and TC-7 cells relative to the expression level in LS180 cells. The MDR1 transcriptional expression was significantly higher in Caco-2 and TC-7 cells compared with LS180 cell line whereas CYP3A4 was expressed more in the LS180 cell line than in Caco-2 and TC-7 cells. Data represent mean$\pm$s.e.m. of n = 3 experiments. $^{**}P < 0.001$.

155-fold compared with LS180 (Figure 2). The CYP3A4 expression level in all three untreated cell lines was very low. In Caco-2 and TC-7 the mRNA level was 4- and 9-times lower, respectively, compared with LS180 (Figure 2).

hPXR expression

hPXR mRNA was expressed in all three cell lines (Figure 3). In LS180 cells the expression level was approximately 5-times higher compared with Caco-2 and TC-7 cells. In Caco-2 and TC-7 cells hPXR transcriptional expression was not altered under treatment with rifampicin or $1,25(OH)_2D_3$, whereas in LS180 cells, hPXR mRNA was significantly decreased after $1,25(OH)_2D_3$ treatment.

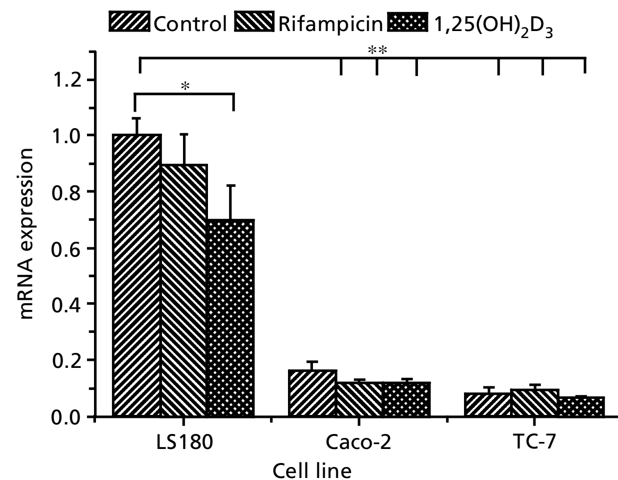

Figure 3 Relative mRNA expression of hPXR mRNA in LS180, Caco-2 parental and TC-7 cells as determined by relative real time PCR. All mRNA expression levels are shown relative to LS180 control mRNA expression. This graph shows a significant higher level of hPXR transcripts in the LS180 cell line compared with Caco-2 and TC-7 cells. hPXR transcriptional expression in LS180 cells with $1,25(OH)_2D_3$ treatment seemed to be lower compared with the control group. Rifampicin had no effect on the transcriptional expression of hPXR in this cell line. Data represent mean$\pm$s.e.m. of n = 3 experiments. $^{*}P < 0.05$, $^{**}P = < 0.001$.

Rank order of ABC-transporters expression

The mRNA expression pattern of several ABC-transporters in the three cell lines were compared with literature data received from human jejunal biopsies, where the rank order was MRP2 > MDR1 ≈ MRP3 ≈ MRP5 ≈ MRP1 ≈ MRP4 (Taipalensuu et al 2001). For Caco-2 and TC-7 cells the expression pattern we obtained was similar to Taipalensuu et al (2001). The rank order we obtained for the ABC-transporter transcripts in Caco-2 cells was MRP3 ≈ MRP2 ≈ MDR1 > MRP5 > MRP1 > MRP4. For TC-7 cells the ranking was MRP3 > MDR1 > MRP2 > MRP5 > MRP1 > MRP4. Thus, the expression pattern of the ABC-transporters in Caco-2 parental and TC-7 cells was similar to human jejunal biopsies, whereas in the LS180 cells, the rank order was clearly different from the ex-vivo data (MRP3 > MRP1 > MRP5 > MDR1 > MRP4 > MRP2). The most striking difference was seen with MRP2 transcriptional expression. In Caco-2 parental, TC-7 and jejunal tissue MRP2 mRNA expression was at the top of the rank order whereas in the LS180 cell line MRP2 transcripts were least detected.

Discussion

MDR1 and CYP3A4 induction

From in-vivo and in-vitro studies it is known that drugs such as rifampicin and St John's Wort can up regulate MDR1 expression (Greiner et al 1999; Durr et al 2000). Local P-gp induction in the epithelium of the gut wall was

identified to be an underlying mechanism of drug interactions between rifampicin and P-gp substrates, such as digoxin (Greiner et al 1999) and fexofenadine (Hamman et al 2001), resulting in a decreased bioavailability of the orally administered drugs.

CYP3A4 is present also in the gastrointestinal tract and, likewise, is inducible by rifampicin. Available information suggests that such prehepatic metabolism can substantially contribute to the total clearance of CYP3A4 substrates (Kolars et al 1992), for example verapamil (Fromm et al 1996) and simvastatin (Kyrklund et al 2000).

In our study, CYP3A4 and MDR1 were induced by rifampicin and 1,25$(OH)_2D_3$ in LS180 cells. These findings together with the observation that other hPXR ligands can induce these two genes in the LS 180 cell line (Schuetz et al 1996) led us to the conclusion that this cell line was a suitable model to study MDR1 and CYP3A4 induction. Schmiedlin-Ren et al (2001) showed that in Caco-2 parental cells, CYP3A4 was induced by 1,25$(OH)_2D_3$ but not by rifampicin. For the Caco-2 subclone TC-7 we could confirm these findings. Only recently has it been documented that hPXR was a key regulator not only for CYP3A4 but also for MDR1 induction (Lehmann et al 1998; Geick et al 2001). The fact that the PXR ligand rifampicin could induce neither CYP3A4 nor MDR1 in Caco-2 or TC-7 cells led to the conclusion that hPXR was not active in these cells. Thus the Caco-2 parental cell line would not be a valid model to study MDR1 and CYP3A4 induction.

1,25$(OH)_2D_3$ was a potent inducer of CYP3A4 in all three cell lines and also of MDR1 in LS180 cells. However, it was not completely clear whether 1,25$(OH)_2D_3$ activated hPXR or whether 1,25$(OH)_2D_3$ induced CYP3A4 and MDR1 via another transcriptional regulator. Schmiedlin-Ren et al (2001) suggested that the vitamin D receptor, a nuclear receptor known to be expressed in Caco-2 cells, could mediate this induction. They showed also that 25-OH-D_3 and 1-OH-D_3, two known vitamin D receptor ligands, were able to induce CYP3A4. This finding would support the role of the vitamin D receptor in 1,25$(OH)_2D_3$-mediated CYP3A4 induction.

The mechanism of this induction was elucidated by Thummel et al (2001) who proposed that 1,25$(OH)_2D_3$ promoted CYP3A4 gene transcription by binding to the vitamin D receptor. When this complex heterodimerized with RXR, it bound specifically to the hPXR response element in the CYP3A4 promoter region.

MRP1–MRP5 expression

Apart from the multidrug resistance protein MDR1, many other more recently discovered efflux proteins of the ATP-binding cassette super family might influence the pharmacokinetics, tissue distribution and pharmacodynamics of drugs (Ambudkar et al 1999; Borst et al 2000). Here we found that MRP1, MRP2, MRP3, MRP4 and MRP5 were expressed in parental Caco-2, TC-7 and LS180 cells. MRP2 to MRP5 expression was significantly higher in Caco-2 parental and TC-7 cells compared with LS180 cells. The MRP expression pattern in Caco-2 parental and TC-7 cell lines seemed to correlate quite well with the expression in human jejunum (Taipalensuu et al 2001). These findings, together with the observation that MDR1 mRNA levels in LS180 cells were much lower compared with the Caco-2 cells, led us to conclude that the Caco-2 parental and TC-7 cells would represent an appropriate model to study the permeability of the small intestine rather than LS180 cells.

Up to now there has been no evidence to convince us that these transport proteins were determinants for drug disposition. Therefore the influence of these transporter proteins on absorption of orally administered drugs remains to be investigated. As they transport therapeutically important drugs such as methotrexate and pravastatin they might also contribute to drug disposition.

ABC-transporter expression pattern

The expression pattern of the ABC-transporter transcripts in Caco-2 and TC-7 cells investigated in this study was similar to that of human jejunal tissue (Taipalensuu et al 2001). This observation corresponded to the functional similarity of Caco-2 cells and small intestinal enterocytes. In the LS180 cell line the rank order of ABC-transporter mRNA expression levels was completely different and was similar to the one we found in human colonic tissue (data not shown). This suggested that the LS180 cells conserved more properties of their colonic origin.

We concluded that Caco-2 parental and TC-7 cells represented a more appropriate in-vitro model to study ABC-transporter mediated drug transport than the LS180 cell line.

hPXR

In this study we demonstrated that hPXR was expressed in each of the three cell lines. These results were obtained with both classical real time-PCR and TaqMan analysis. In the latter, the relative expression in LS180 cells was approximately 5-fold higher than in the Caco-2 parental and TC-7 cell lines. These results are partly in contrast to the findings of other groups (Thummel et al 2001), who detected hPXR mRNA in LS180 cells but not in Caco-2 parental cells. These apparent differences in the Caco-2 parental cells may be explained by the development of diverse subclones. Various groups have shown that during cultivation, the Caco-2 parental cells tend to develop an increasing number of subclones. This may hamper the comparison of results between different laboratories.

Our results indicated that rifampicin (an hPXR activator) was not able to induce CYP3A4 and MDR1 gene expression in Caco-2 and TC-7 cells (see Figure 1). These observations suggested that hPXR was not functional in these cells. Furthermore, previous work from Wolbold et al (2001) showed that in man the constitutive hPXR expression level did not correlate with the extent of MDR1 or CYP3A4 induction after rifampicin pretreatment.

MDR1 and CYP3A4 induction seems to be a complex process that involves additional factors apart from hPXR

and RXR. One of these additional factors might be human alpha glucocorticoid receptor (hαGR). Pascussi et al (2000) suggested that this receptor was involved in CYP3A4 gene induction by cross-talking with hPXR. Other authors have shown that hαGR was not essential for CYP3A induction, as mice lacking the glucocorticoid receptor did not show any differences in constitutive hepatic expression of CYP3A. Furthermore, rifampicin was able to induce CYP3A in wild type and in glucocorticoid receptor-knockout mice (Schuetz et al 2000). Results from our laboratory have indicated that hαGR is not essential for transcriptional regulation of MDR1 and CYP3A4 because the LS180 cell line, in which MDR1 and CYP3A4 can be induced, lacks hαGR receptor mRNA expression (data not shown).

Conclusion

This study demonstrated that CYP3A4 and MDR1 gene expression was inducible with rifampicin and $1,25(OH)_2D_3$ in the LS180 cell line. The ABC-transporters MDR1 and MRP1–MRP5 in Caco-2 and TC-7 cells showed a similar expression pattern as in human jejunal biopsies. We concluded that LS180 cells were a suitable model to study MDR1 and CYP3A4 induction, but for drug transport studies Caco-2 cells would be preferred as the more physiological model.

References

Ambudkar, S. V., Dey, S. C., Hrycyna, A., Ramachandra, M., Pastan, I., Gottesman, M. M. (1999) Biochemical, cellular, and pharmacological aspects of the multidrug transporter. *Annu. Rev. Pharmacol. Toxicol.* **39**: 361–398

Anderson, J. M., Van Itallie, C. M., Peterson, M. D., Stevenson, B. R., Carew, E. A., Mooseker, M. S. (1989) ZO-1 mRNA and protein expression during tight junction assembly in Caco- 2 cells. *J. Cell Biol.* **109**: 1047–1056

Borst, P., Evers, R., Kool, M., Wijnholds, J. (1999) The multidrug resistance protein family. *Biochim. Biophys. Acta* **1461**: 347–357

Borst, P., Evers, R., Kool, M., Wijnholds, J. (2000) A family of drug transporters: the multidrug resistance-associated proteins. *J. Natl Cancer Inst.* **92**: 1295–1302

Chantret, I., Rodolosse, A., Barbat, A., Dussaulx, E., Brot-Laroche, E., Zweibaum, A., Rousset, M. (1994) Differential expression of sucrase-isomaltase in clones isolated from early and late passages of the cell line Caco-2: evidence for glucose-dependent negative regulation. *J. Cell Sci.* **107**: 213–225

Durr, D., Stieger, B., Kullak-Ublick, G. A., Rentsch, K. M., Steinert, H. C., Meier, P. J., Fattinger, K. (2000) St John's Wort induces intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4. *Clin. Pharmacol. Ther.* **68**: 598–604

Engman, H. A., Lennernas, H., Taipalensuu, J., Otter, C., Leidvik, B., Artursson, P. (2001) CYP3A4, CYP3A5, and MDR1 in human small and large intestinal cell lines suitable for drug transport studies. *J. Pharm. Sci.* **90**: 1736–1751

Fogh, J., Wright, W. C., Loveless, J. D. (1977) Absence of HeLa cell contamination in 169 cell lines derived from human tumors. *J. Natl Cancer Inst.* **58**: 209–214

Fromm, M. F., Busse, D., Kroemer, H. K., Eichelbaum, M. (1996) Differential induction of prehepatic and hepatic metabolism of verapamil by rifampin. *Hepatology* **24**: 796–801

Geick, A., Eichelbaum, M., Burk, O. (2001) Nuclear response elements mediate induction of intestinal MDR1 by rifampin. *J. Biol. Chem.* **31**: 31

Greiner, B., Eichelbaum, M., Fritz, P., Kreichgauer, H. P., von Richter, O., Zundler, J., Kroemer, H. K. (1999) The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. *J. Clin. Invest.* **104**: 147–153

Gres, M. C., Julian, B., Bourrie, M., Meunier, V., Roques, C., Berger, M., Boulenc, X., Berger, Y., Fabre, G. (1998) Correlation between oral drug absorption in humans, and apparent drug permeability in TC-7 cells, a human epithelial intestinal cell line: comparison with the parental Caco-2 cell line. *Pharm. Res.* **15**: 726–733

Gutmann, H., Fricker, G., Torok, M., Michael, S., Beglinger, C., Drewe, J. (1999) Evidence for different ABC-transporters in Caco-2 cells modulating drug uptake. *Pharm. Res.* **16**: 402–407

Hamman, M. A., Bruce, M. A., Haehner-Daniels, B. D., Hall, S. D. (2001) The effect of rifampin administration on the disposition of fexofenadine. *Clin. Pharmacol. Ther.* **69**: 114–121

Hirohashi, T., Suzuki, H., Chu, X. Y., Tamai, I., Tsuji, A., Sugiyama, Y. (2000) Function and expression of multidrug resistance-associated protein family in human colon adenocarcinoma cells (Caco-2). *J. Pharmacol. Exp. Ther.* **292**: 265–270

Kim, R. B., Wandel, C., Leake, B., Cvetkovic, M., Fromm, M. F., Dempsey, P. J., Roden, M. M., Belas, F., Chaudhary, A. K., Roden, D. M., Wood, A. J., Wilkinson, G. R. (1999) Interrelationship between substrates and inhibitors of human CYP3A and P-glycoprotein. *Pharm. Res.* **16**: 408–414

Kolars, J. C., Schmiedlin-Ren, P., Schuetz, J. D., Fang, C., Watkins, P. B. (1992) Identification of rifampin-inducible P450IIIA4 (CYP3A4) in human small bowel enterocytes. *J. Clin. Invest.* **90**: 1871–1878

Kosel, B. W., Aweeka, F. (2000) Drug interactions of antiretroviral agents. *AIDS Clin. Rev.* **01**: 193–227

Krishna, D. R., Klotz, U. (1994) Extrahepatic metabolism of drugs in humans. *Clin. Pharmacokinet.* **26**: 144–160

Kyrklund, C., Backman, J. T., Kivisto, K. T., Neuvonen, M., Laitila, J., Neuvonen, P. J. (2000) Rifampin greatly reduces plasma simvastatin and simvastatin acid concentrations. *Clin. Pharmacol. Ther.* **68**: 592–597

Lehmann, J. M., McKee, D. D., Watson, M. A., Willson, T. M., Moore, J. T., Kliewer, S. A. (1998) The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J. Clin. Invest.* **102**: 1016–1023

Pascussi, J. M., Drocourt, L., Fabre, J. M., Maurel, P., Vilarem, M. J. (2000) Dexamethasone induces pregnane X receptor and retinoid X receptor-alpha expression in human hepatocytes: synergistic increase of CYP3A4 induction by pregnane X receptor activators. *Mol. Pharmacol.* **58**: 361–372

Schmiedlin-Ren, P., Thummel, K. E., Fisher, J. M., Paine, M. F., Lown, K. S., Watkins, P. B. (1997) Expression of enzymatically active CYP3A4 by Caco-2 cells grown on extracellular matrix-coated permeable supports in the presence of 1alpha,25-dihydroxy-vitamin D3. *Mol. Pharmacol.* **51**: 741–754

Schmiedlin-Ren, P., Thummel, K. E., Fisher, J. M., Paine, M. F., Watkins, P. B. (2001) Induction of cyp3a4 by 1alpha,25-dihydroxy-vitamin d(3) is human cell line-specific and is unlikely to involve pregnane x receptor. *Drug Metab. Dispos.* **29**: 1446–1453

Schuetz, E. G., Schinkel, A. H., Relling, M. V., Schuetz, J. D. (1996) P-glycoprotein: a major determinant of rifampicin-inducible expression of cytochrome P4503A in mice and humans. *Proc. Natl Acad. Sci. USA* **93**: 4001–4005

Schuetz, E. G., Schmid, W., Schutz, G., Brimer, C., Yasuda, K.,

Kamataki, T., Bornheim, L., Myles, K., Cole, T. J. (2000) The glucocorticoid receptor is essential for induction of cytochrome P-4502B by steroids but not for drug or steroid induction of CYP3A or P-450 reductase in mouse liver. *Drug Metab. Dispos.* **28**: 268–278

Synold, T. W., Dussault, I., Forman, B. M. (2001) The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. *Nat. Med.* **7**: 584–590

Taipalensuu, J., Tornblom, H., Lindberg, G., Einarsson, C., Sjoqvist, F., Melhus, H., Garberg, P., Sjostrom, B., Lundgren, B., Artursson, P. (2001) 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. *J. Pharmacol. Exp. Ther.* **299**: 164–170

Thummel, K. E., Brimer, C., Yasuda, K., Thottassery, J., Senn, T., Lin, Y., Ishizuka, H., Kharasch, E., Schuetz, J., Schuetz, E. (2001) Transcriptional control of intestinal cytochrome P-4503A by 1alpha,25-dihydroxyvitamin D(3). *Mol. Pharmacol.* **60**: 1399–1406

Wacher, V. J., Wu, C. Y., Benet, L. Z. (1995) Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. *Mol. Carcinog.* **13**: 129–134

Watkins, P. B. (1997) The barrier function of CYP3A4 and P-glycoprotein in the small bowel. *Adv. Drug Deliv. Rev.* **27**: 161–170

Wolbold, R., Eichelbaum, M., Burk, O. (2001) Induction of intestinal CYP3A4 and MDR1 by rifampin is polymorphic and coordinately regulated. *Drug Metab. Rev.* **33**: 155

Wrighton, S. A., Schuetz, E. G., Thummel, K. E., Shen, D. D., Korzekwa, K. R., Watkins, P. B. (2000) The human CYP3A subfamily: practical considerations. *Drug Metab. Rev.* **32**: 339–361

Zarr, J. H. (1984) *Biostatistical analysis.* Prentice Hall, Inc. Englewood Cliffs, NJ, US

Zhang, Y., Benet, L. Z. (2001) The gut as a barrier to drug absorption: combined role of cytochrome P450 3A and P-glycoprotein. *Clin. Pharmacokinet.* **40**: 159–168