Interplay Between CYP3A-Mediated Metabolism and Polarized Efflux of Terfenadine and Its Metabolites in Intestinal Epithelial Caco-2 (TC7) Cell Monolayers

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Purpose. To further characterize cytochrome P450 (CYP) and P-glycoprotein (Pgp) expression in monolayers of the Caco-2 cell clone TC7, a cell culture model of the human intestinal epithelium. To study the interplay between CYP3A and Pgp as barriers to intestinal drug absorption in TC7 cells using terfenadine and its metabolites as substrates.

Methods. mRNA expression of eight CYPs and Pgp was investigated in TC7 and parental Caco-2 (Caco-2p) cell monolayers using RT-PCR. The CYP3A kinetics was determined in microsomes from both cell lines. The transport, metabolism and efflux of terfenadine and its metabolites were investigated in TC7 monolayers.

Results. Both TC7 and Caco-2p cells expressed mRNA for Pgp and several important CYPs. However, mRNA for CYP3A4 was detectable only from TC7 cells. The relative affinity of CYP3A for terfenadine metabolism in the two cell lines was comparable, but the maximum reaction rate in the TC7 cells was 8-fold higher. The rate of transport of terfenadine and its metabolites hydroxy-terfenadine (HO-T) and azacyclonol across TC7 monolayers was 7.1-, 3.5- and 2.1-fold higher, respectively, in the basolateral to apical direction than it was in the apical to basolateral (AP-BL) direction. Inhibition studies indicated that the efflux was mediated by Pgp. Ketoconazole increased the AP-BL transport terfenadine dramatically by inhibiting both terfenadine metabolism and Pgp efflux.

Conclusions. Cell culture models such as TC7 provide qualitative information on drug interactions involving intestinal CYP3A and Pgp.

KEY WORDS: TC7; Caco-2; cytochrome P450; CYP3A; terfenadine; intestinal metabolism.

INTRODUCTION

Intestinal cytochrome P450s (CYPs) and P-glycoprotein (Pgp) are recognized as potentially important barriers to drug absorption and hence oral bioavailability. CYP3A4 is the major CYP expressed in the small intestine; it metabolizes more than 50% of all drugs (1,2). The poor oral bioavailability of drugs such as cyclosporin and midazolam have been partly attributed to intestinal CYP3A-mediated drug metabolism (3,4). Interestingly, there is a large overlap in the substrate specificity of CYP3A4 and Pgp (5). Studies in Pgp-knockout mice indicate that Pgp may be a limiting factor in the oral bioavailability of some drugs (6). For example, the bioavailability of paclitaxel (Taxol), indinavir and nelfinavir was higher in Pgp-knockouts than in wild type mice (7,8). However, studies in man have not yet provided conclusive evidence for Pgp as a barrier to intestinal drug absorption (9,10). Despite the large substrate overlap between CYP3A and Pgp, the potential interplay between these two intestinal barriers has not been explored in detail.

Initial mechanistic studies on this interplay could be performed under controlled conditions in *in vitro* models such as monolayers of Caco-2 cells. Unfortunately, the drug metabolizing enzymes in Caco-2 cell monolayers are relatively poorly characterized. Expression of some, but not all, of the intestinal CYPs have been investigated in this cell line (11–13). Thus, there is a need to further characterize CYP expression in Caco-2 cells. Recently, a monolayer-forming subclone of the parental Caco-2 cell line (Caco-2p), named TC7, was found to overexpress CYP3A as compared to the Caco-2p cells (13). Biochemical and functional characterization showed that mainly CYP3A5 rather than CYP3A4 is expressed in TC7 cells (14,15).

In this study, we extend the characterization of the drug metabolizing capacity of Caco-2 cells by comparing the mRNA expression of eight CYPs in TC7 and Caco-2p cells with that in the human intestine and colon. We also compare the kinetics of CYP3A metabolism in these cell lines using terfenadine as a model drug. We then investigate the interaction of terfenadine and its metabolites with CYP3A and Pgp. Terfenadine was chosen as a model drug in this study since its oral bioavailability in humans is $<1\%$ as a result of extensive CYP3A metabolism, and possibly, efflux mechanism. About 40% of an orally administered dose of terfenadine is detected in the urine as metabolites while the remaining 60% is found as metabolites in the faeces (16). The mechanisms of elimination are of profound importance since increased plasma levels of terfenadine are known to be cardiotoxic (17). This cardiotoxicity recently lead to voluntarily discontinued distribution and marketing of all terfenadine-containing antihistamine product lines in the United States.

MATERIALS AND METHODS

Materials

Terfenadine was purchased from Sigma Chemical Co. (St. Louis, MO). Terfenadine metabolites hydroxy-terfenadine (HOT) and azacyclonol (AC) were obtained from Phoenix International (Montreal, Quebec, Canada). Ketoconazole was obtained from Research Diagnostics (Flander, NJ). The Caco-2 cells were obtained from American Tissue Culture Collection (ATCC; Rockville, MD, U.S.A.). The Caco-2-TC7 cell line (TC7) was a generous gift from Dr. Alain Zweibaum (INSERM U178, Cedex, France). Rat tail collagen (Type I) was purchased from Collaborative Research (Bedford, MA). Dulbecco's modified Eagle's medium (DMEM) was obtained from Gibco (Grand Island, NJ). Fetal bovine serum (FBS), penicillin-streptomycin solution, trypsin (0.25%) EDTA (1 mM), non-essential amino

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ABBREVIATIONS: CYP, cytochrome P450; Pgp, p-glycoprotein (MDR-1); RT-PCR, reverse transcription polymerase chain reaction; DMEM, Dulbecco's modified Eagle's medium; NEAA, amino acids; HBSS, Hank's balanced salts solution; AP, apical; BL, Non-essential basolateral; AP-BL, apical to basolateral.

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acids (NEAA, 100X), L-glutamine (200 mM), Dulbecco's phosphate buffered saline (DPBS), and Hank's balanced salt solution (HBSS) were obtained from JRH Bioscience (Lenexa, KS). Transwell™ clusters, 24.5 mm in diameter and with 0.4 μm pore size, were from Costar Corp. (Bedford, MA).

METHODS

Cell Culture

TC7 and Caco-2 cell cultures were maintained in flasks in an atmosphere of 5% CO_2 and 90% relative humidity. TC7 cells were seeded onto collagen-coated polycarbonate Transwell™ inserts at a density of 60,000 cells/cm^2. The cells were cultured for 15–20 days in DMEM supplemented with 20% fetal bovine serum, 1% non-essential amino acids, 1% L-glutamine, penicillin (100 units/ml) and streptomycin (100 μg/ml). TC7 and Caco-2 cells were used between passages 25–50 and 98–105, respectively. The integrity of the cell monolayers was determined by measuring the transepithelial transport of the integrity marker, Lucifer yellow. The rate of Lucifer yellow transport in TC7 cells was 0.023%/hr.

Reverse Transcription Polymerase Chain Reactions (RT-PCR)

Total RNA was isolated from 5 × 10^6 Caco-2 or TC7 cells using the Rneasy Midi kit (Quiagen, Hilden, Germany). cDNA was synthesized using 1 μg total RNA, 50 U Taq polymerase, dNTP's and 30 pmol oligo T (15 bp). The PCR for CYPs 2C8-19, 2E1, 3A4, and 3A7, Pgp and actin was performed in PCR buffer containing 3 mM $MgCl_2$, dNTP's and 30 pmol primers according to the following: 1 min at 94°C, 15 s at 55°C, 45 s at 72°C, 30 cycles of 15 s each at 94°C, 15 s at 55°C and 45 s at 72°C, and 10 min at 72°C, respectively. The PCR for CYPs 1A1, 2A6, 2B6, 2D6, 3A5 was performed as described above but with an annealing temperature of 50°C. The primers and size of the PCR product are listed in Table I. The primers were obtained from T-A-G-Copenhagen (Copenhagen, Denmark).

Microsome Preparation and Enzyme Assay

TC7 and Caco-2 microsomes were prepared as reported previously (15). The enzyme kinetics determined as described previously, using terfenadine as a substrate (15).

Transport and Metabolism Studies in TC7 Cells

Cell monolayers were washed with prewarmed HBSS (pH 7.4). HBSS containing either terfenadine (20 μM), HO-T (20 μM) or AC (38 μM) was applied to the apical (AP) or basolateral (BL) side of the monolayers. Samples from the donor chambers were taken at the start of the experiments. When the drug was added to the AP side, the insert was moved to a well with fresh buffer every 60 min. A 0.5 ml aliquot was removed from each well for HPLC analysis. When the drug was added to the BL compartment, an aliquot (0.5 ml) was removed from the upper compartment every 60 min and the buffer in the insert was replaced with 0.5 ml fresh HBSS. At the end of the experiment, aliquots were taken from each side of the monolayers. Ketoconazole (100 μM) or digoxin (50 μM) were used to inhibit terfenadine metabolism and transport. Verapamil (100 μM), HO-T (100 μM), AC (100 μM) and ketoconazole (100 μM) were used to inhibit digoxin transport.

In the studies of Pgp inhibition, HBSS containing ^{3}H-digoxin was added to the AP side of the monolayers, while the inhibitors (100 μM) were added to both AP and BL compartments. The transport of ^{3}H-digoxin was followed for 90 min. The inserts were moved to new wells with fresh buffer every 30 min. Samples were taken from the BL compartment and measured in a Liquid Scintillation Counter.

When the transport and metabolism of terfenadine were followed simultaneously, HBSS containing 20 μM of terfenadine was added to the AP or BL side of the cell monolayers and the transport and metabolism of the drug was followed for

Table I. Oligonucleotide PCR Primers for Human CYP Isoenzymes, Pgp, and Actin

Enzyme	Primer		Fragment (bp)
CYP 1A1	A	5′TCACAGACAGCCTGATTGAGCAC 3′	432
	B	5′GATGGGTTGACCCATAGCTT 3′	
CYP2B6	A	5′CCATACACAGAGGCAGTCAT 3′	357
	B	5′AGGTGTCAGATCGATGTCTTC 3′	
CYP 2D6	A	5′TGATGAGAACCTGCGCATAG 3′	332
	B	5′ACCGATGACAGGTTGGTGAT 3′	
CYP2E1	A	5′CTGCAACGTCATAGCCGACA 3′	297
	B	5′TCCATTTCCACGAGCAGGCA 3′	
CYP 2C8-19	A	5′GCTAAAGTCCAGGAAGAGATTGA 3′	332
	B	5′TCCTGCTGAGAAAGGCATGAAGT 3′	
CYP 3A4	A	5′CATACACACCCTTTGGAAGT 3′	367
	B	5′ATGTACAGAATCCCCGGTTA 3′	
CYP 3A5	A	5′TGTCCAGCAGAAACTGCAAA 3′	470
	B	5′TTGAAGAAGTCCTTGCGTGTC 3′	
CYP 3A7	A	5′TCAGGCTCCACTTACGGTCT 3′	484
	B	5′CCACCTATGATACTGTGCTACAGTT 3′	
Pgp	A	5′GAGCGCGAGGTCGGGATGGATCTTGAAGGGGA 3′	286
	B	5′TTGACATCAGATCTTCTAAATTTCCTGCATTT 3′	
Actin	A	5′GTGGGGCGCCCCAGGCACCA 3′	541
	B	5′CTCCTTAATGTCACGCACGATTTC 3′	

180 min. When terfenadine was added to the AP side, the inserts were moved to a well with fresh buffer every 60 min. Aliquots (0.5 ml) were taken from the AP and BL sides every 60 min as described above. The cumulative transport of terfenadine and the metabolites was calculated after correction for dilution. Samples from donor solutions were always taken prior to the experiments. Transport across the TC7 monolayers was expressed as pmol/cm^2*min. The surface area of the cell monolayers was 4.71 cm^2. The values are means ±s.d. Mean values were compared using the Unpaired Students t-test or analysis of variance (ANOVA) when the number of groups were more than two.

HPLC Analysis

The HPLC system consisted of a Waters Powerline 600 controller, a Waters 710 WISP autoinjector, a CYANO 5 μm, 4.6 × 25 cm column and a Hitachi L-1050 fluorescence detector. The temperature of the column was maintained at 35°C. Excitation and emission wavelengths were set at 230 nm and 280 nm, respectively. Data were collected using Nelson data acquisition software. The mobile phase consisted of acetonitrile/methanol/12 mM ammonium acetate buffer pH 4.3 (15/22/63, v/v/v) with a flow rate of 1.3 ml/min. Retention times of terfenadine and its metabolites were confirmed by comparison with retention times of the corresponding standards. Terfenadine, HO-T and AC were quantified using standard curves of peak area versus drug concentration. The limit of detection for both terfenadine and HO-T was 10 pmol/ml.

RESULTS

Expression of mRNA for CYP and Pgp

Both TC7 and Caco-2 cells expressed mRNA for several important intestinal CYPs, including CYP 1A1, 2C8-19, 2E1, and 3A5, and also for Pgp (Fig. 1). In addition, TC7 cells expressed mRNA for CYP3A4 while Caco-2 cells expressed mRNA for CYPs 2B6 and 2D6. Thus, different clones of Caco-2 cells displayed different patterns of CYP mRNA. There were also some differences between expression in the cell lines and expression in the human intestine and colon (Table II). However, additional studies on CYP expression at the protein level and enzymatic activity are required before any firm conclusions regarding CYP expression in these cell lines can be drawn.

CYP3A Catalytic Activity

The catalytic activity of CYP3A in both cell lines, using terfenadine as a substrate, is shown in Table III. While the relative affinity of CYP3A for terfenadine (K_m) was comparable between the cell lines, the maximal reaction rate (V_{max}) was about 8-fold higher in TC7 cells (15). TC7 cells were thus selected for further studies of CYP3A and Pgp as barriers to drug transport.

Transport of Terfenadine, HO-T and AC

The transport of terfenadine, HO-T and AC was studied in both directions across TC7 monolayers. The rates of AP-BL flux were 0.21 pmol/cm^2 * min, 4.2 pmol/cm^2 * min, and 12 pmol/cm^2 * min, respectively. Thus, the TC7 monolayers were >20-fold more permeable to the added metabolites than to terfenadine. The rate of BL-AP flux for terfenadine was 7.1-fold higher than that in the AP-BL direction ($p < 0.0001$), suggesting that terfenadine was actively secreted across the TC7 cells. Similar, but somewhat less pronounced, results were observed for HO-T and AC (Table IV). Thus, HO-T and AC displayed a 3.5-fold and 2.1-fold higher rate of transport in the secretory direction.

Transport of Terfenadine and Formation and Transport of HO-T

Terfenadine (20 μM) was added to the AP or BL side of the TC7 monolayers and the transport of terfenadine and the formation and transport of HO-T were followed. The transport of terfenadine and its metabolite increased linearly with time (Fig. 2). In agreement with the results in Table IV, the rate of transport of terfenadine and HO-T was higher in the BL-AP direction than in the AP-BL direction (Fig. 2). However, the rate of flux of HO-T formed from terfenadine was much lower in both directions than that observed in the HO-T transport experiments shown in Table IV (Table V). These results indicate that HO-T formation was the rate-limiting step in the transport of HO-T through TC7 cells.

The transport of HO-T was also determined after incubation of terfenadine (20 μM) on either the AP or BL side of the TC7 monolayers. Regardless of the side to which terfenadine was added, most of the HO-T formed by CYP3A was recovered in the AP compartment, indicating a preferential release of HO-T on the AP side of TC7 cell monolayers.

Effect of Pgp Inhibition

The effect of digoxin (50 μM) on HO-T and terfenadine flux was studied in order to investigate whether their secretion by TC7 cells was mediated by Pgp. Digoxin is a Pgp substrate which is not metabolized by CYP3A in human subjects (21,22). Digoxin had a clear effect on terfenadine and HO-T transport: the rate of flux of terfenadine was increased by 39% ($P < 0.05$) and that of HO-T was increased by 70% ($P < 0.05$) (Fig. 3). The highest concentration of digoxin that could be used was 50 μM, because of its low solubility in HBSS.

Cross inhibition studies, using ^{3}H-digoxin as a substrate, were also performed (Fig. 4). Unfortunately, terfenadine affected the integrity of the cell monolayers at inhibitory concentrations, as indicated by a large increase in Lucifer yellow flux (data not shown), and inhibition studies with this molecule could not be undertaken. The transport of ^{3}H-digoxin in the AP-BL direction increased 15-fold in the presence of 100 μM of HO-T ($p < 0.05$), while only a small increase in ^{3}H-digoxin was observed when the other terfenadine metabolite, AC, was used as an inhibitor ($p < 0.05$). By comparison, the Pgp substrate verapamil increased the flux of ^{3}H-digoxin 7-fold ($p < 0.05$; Fig. 4). Together, the inhibition studies indicate that Pgp is involved in the transport of terfenadine, HO-T and AC in TC7 cells.

Effect of CYP3A Inhibition

The influence of CYP3A metabolism on the transport of terfenadine and HO-T was investigated using ketoconazole, an inhibitor of CYP3A. Ketoconazole (100 μM) completely

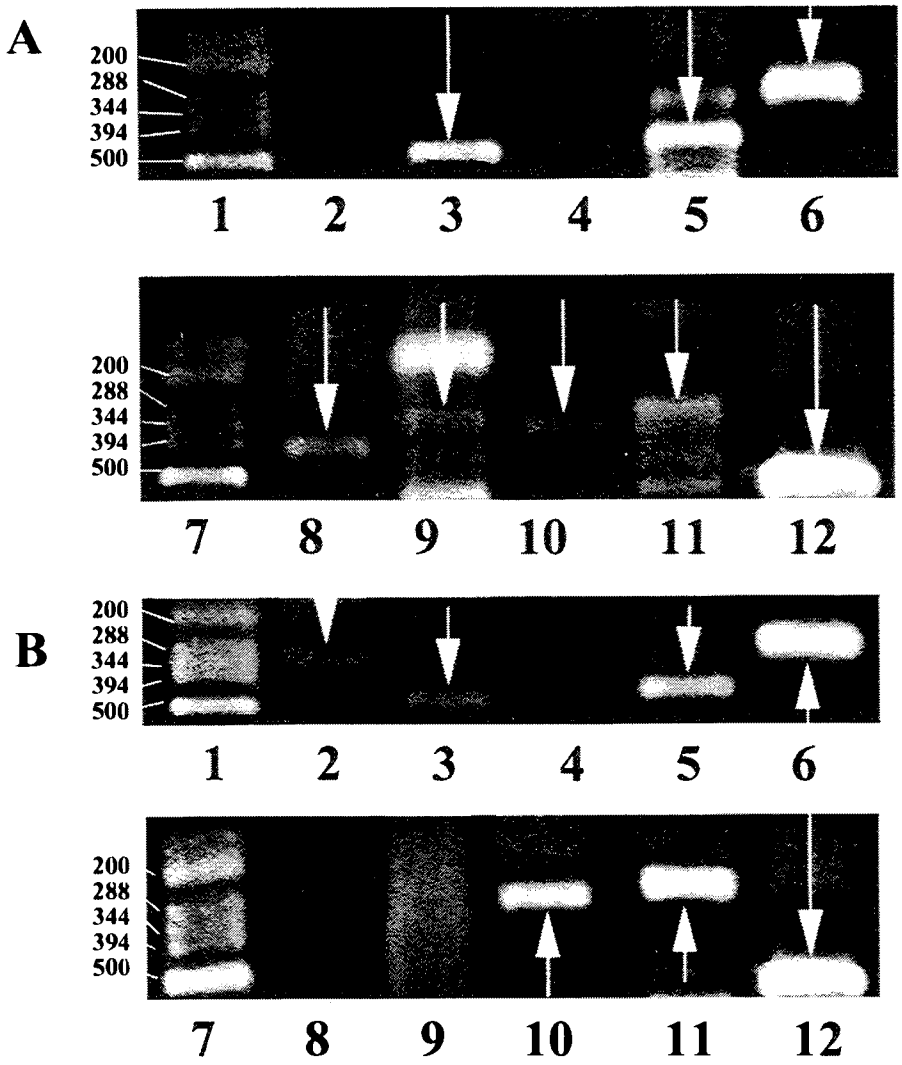

Fig. 1. Qualitative expression of mRNA for eight CYPs and Pgp in Caco-2p (A) and TC7 cells (B). A and B: Molecular weight markers (lane 1), CYP3A4 (lane 2), CYP3A5 (lane 3), CYP3A7 (lane 4), CYP1A1 (lane 5), Pgp (lane 6), Molecular weight marker (lane 7), CYP2D6 (lane 8), CYP2B6 (lane 9), CYP2C8-19 (lane 10), CYP2E1 (lane 11), actin (lane 12).

Table II. Comparison of CYP mRNA Expression in Caco-2p and TC7 Cells with that in Human Intestine and Colon

Enzyme	Caco-2p[a]	TC7 cells[a]	Human intestine[b]	Human colon[b]
CYP1A1	+	+	+	+
CYP2B6	+	−		
CYP2C8-19	+	+	+	−
CYP2D6	+	−	+	−
CYP2E1	+	+	+	−
CYP3A4	−	+	+	+
CYP3A5	+	+	+	+
CYP3A7	−	−	−	−

[a] A band of the correct size seen on gel is marked with +. A negative result is marked with −.
[b] Data were taken from Refs. 1, 18-20.

inhibited the formation of HO-T (Fig. 5A). Concomitantly, the transport of terfenadine increased 13-fold ($p < 0.0001$; Fig. 5B). The effect of ketoconazole (100 μM) on the transport of the Pgp substrate ^{3}H-digoxin was also investigated (Fig. 6). Interestingly, ketoconazole increased the flux of ^{3}H-digoxin 18-fold ($p < 0.0001$), suggesting that ketoconazole is a strong inhibitor of Pgp.

Table III. Kinetics of Terfenadine Hydroxylation in Microsomes from TC7 and Caco-2p Cells

Cell	K_m (μM)	V_{max} (pmol/min/mg protein)
TC7[a]	1.9	2.11
Caco-2[b]	1.03	0.26

[a] Data from Ref. 15.
[b] The enzyme kinetics determined as described previously (15). Each point represents the average of duplicates.

Table IV. Transepithelial Flux of Terfenadine, HO-T, and AC Across TC7 Cell Monolayers[a]

Compounds (μM)	AP-BL (pmol/cm²*min)	BL-AP (pmol/cm²*min)	Ratio (BL-AP/AP-BL)
Terfenadine	0.21 ± 0.05	1.5 ± 0.1	7.1
HO-T	4.2 ± 0.2	14 ± 0.4	3.5
AC	12 ± 1	25 ± 1.0	2.1

[a] The rates of AP-BL and BL-AP transport of terfenadine (20 μM), HO-T (20 μM), and AC (38 μM) were determined after 180 min incubation at 37°C. The compounds were added to the donor compartment and their appearance in the receiver compartment was followed with time as described in the Methods section. The transport rates are mean values ± s.d., n = 4.

Table V. Polarized Efflux of HO-T after Terfenadine Hydroxylation by CYP3A in TC7 Cells[a]

	HO-T release		
Addition of terfenadine (20 μM)	AP compartment (pmol/cm²*min)	BL compartment (pmol/cm²*min)	Ratio AP/BL
AP	0.95 ± 0.06	0.32 ± 0.02	3.0
BL	0.98 ± 0.03	0.26 ± 0.02	3.7

[a] Cells were incubated for 180 min at 37°C with terfenadine added to either AP or BL sides of the cell monolayers. The appearance of the formed metabolite, HO-T, was followed with time on the AP or BL sides of the monolayers. The transport rates are means ± s.d., n = 4.

DISCUSSION

In summary, the present investigation identifies TC7 cell monolayers as an *in vitro* model for preliminary studies of the interplay between CYP3A metabolism and Pgp mediated efflux in the intestine. CYP3A metabolism and Pgp mediated efflux have complementary roles in reducing the absorptive flux of terfenadine and its metabolites across the enterocyte. Comparison with human data indicates that results generated in this and in other comparable *in vitro* models are of a qualitative rather than a quantitative nature and should therefore be interpreted with caution.

mRNA for several important drug-metabolizing CYPs of the human intestine was found in both Caco-2p and TC7 cell lines, but none of the cell lines expressed all the relevant CYPs. There are at least two possible explanations for this discrepancy. Firstly, some CYPs are inducible and this study was performed in the absence of specific inducers. Indeed, preliminary data in our laboratory, using a recently published approach (12), indicate that CYP3A4 is inducible in both of these cell lines (23). Secondly, since the Caco-2 cell line was isolated from a colorectal carcinoma, it cannot be expected to be a perfect model of the normal human intestinal or colonic epithelium (24). This

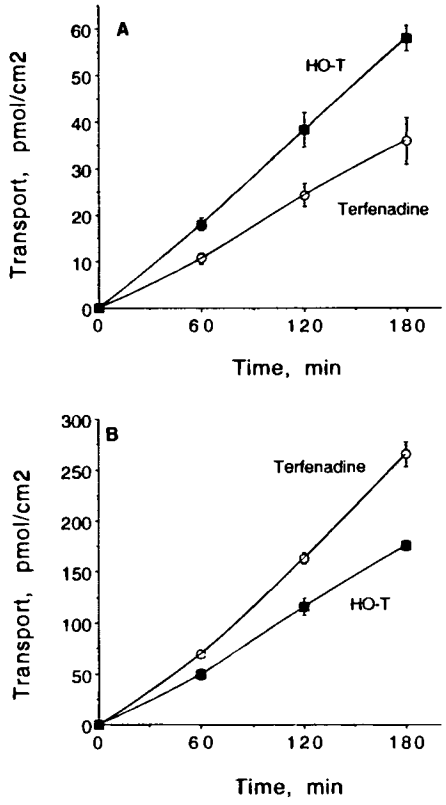

Fig. 2. Transport of terfenadine and HO-T (formed from terfenadine) across TC7 cell monolayers. AP-BL (A), and BL-AP (B) transport of terfenadine (initial concentration 20 μM) and HO-T across TC7 cell monolayers. Values are the mean of four measurements ± s.d.

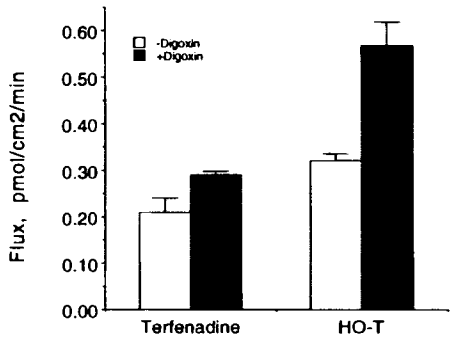

Fig. 3. Effect of the Pgp substrate digoxin (50 μM) on the AP-BL transport of terfenadine (initial concentration 20 μM) and HO-T (formed from terfenadine) across TC7 cell monolayers. Cells were incubated for 180 min with terfenadine alone or terfenadine plus digoxin. Values are the mean of four measurements ± s.d.

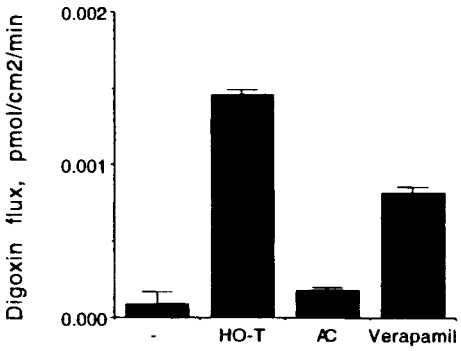

Fig. 4. Effect of 100 μM of HO-T, AC or verapamil on AP-BL transport of ^{3}H-digoxin across TC7 cell monolayers. Values are the mean of four measurements ± s.d.

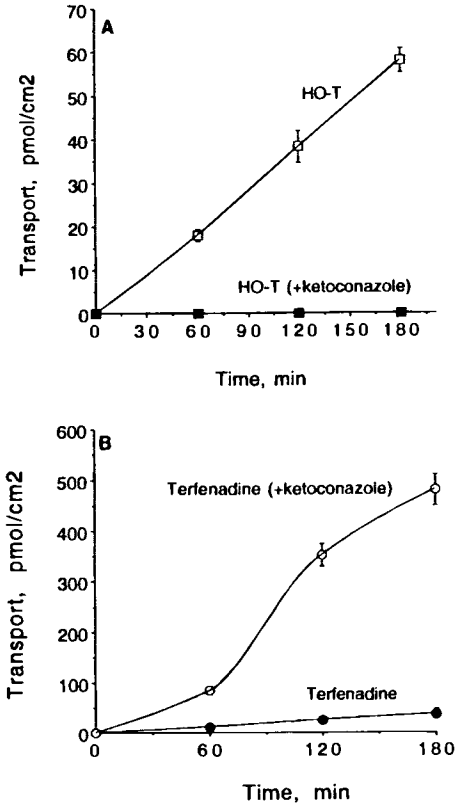

Fig. 5. Effect of ketoconazole on transport of terfenadine and HO-T (formed from terfenadine) across TC7 cell monolayers. A. AP-BL transport of HO-T in presence and absence of 100 μM ketoconazole. B. AP-BL transport of terfenadine (initial concentration 20 μM) was studied in the presence or absence of 100 μM ketoconazole. Values are the mean of four measurements ± s.d.

is also illustrated by the expression pattern of CYP mRNA shown in the cell lines in this paper: the pattern is more comparable to that of the human small intestine than the colon. CYP2C8-19, 2D6 and 2E1 have not been identified previously (at the protein level) in human colonic microsomes (19). Our preliminary data on CYP mRNA expression in the Caco-2p and TC7 cell clones extend previous investigations on CYP expression in intestinal epithelial cell lines (11,12) and provide a platform for further investigations of these enzymes.

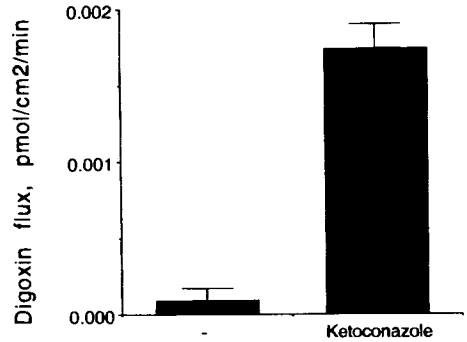

Fig. 6. Effect of ketoconazole (100 μM) on AP-BL transport of ^{3}H-digoxin across TC7 cell monolayers. Values are the mean of four measurements ± s.d.

Although the studies were more of a qualitative than a quantitative nature, the mRNA expression of CYP3A in TC7 cells was surprisingly low compared to that in Caco-2p cells. Since this result was in contradiction to previous studies in these cell lines (13), the enzyme kinetics of Caco-2p were compared with those of TC7 cells, using terfenadine as a substrate. The results showed that the activity (V_{max}) of CYP3A was 8-fold higher in TC7 than in Caco-2p cells. This is in agreement with previous studies on TC7 cells (13) and shows that the level of mRNA expression does not predict the level of protein expression and enzymatic activity. Functional studies on the efflux capacity of Pgp gave comparable results in the two cell lines (data not shown). Together, these results indicate that TC7 cells comprise a more suitable model for studies of the interplay between CYP3A and Pgp than Caco-2p cells.

The finding that terfenadine is a Pgp substrate is supported by previous studies showing that terfenadine inhibits the efflux of doxorubicin, another Pgp substrate, in human breast cancer cells (25). Terfenadine also fulfills the recently discovered structural requirements for interaction with Pgp (26), in that it possesses two electron donor groups at a spatial distance of 2.5 Å.

Pgp reduced the absorptive flux of terfenadine in the TC7 cells. It can be speculated that this efflux mechanism is important in reducing terfenadine bioavailability *in vivo*, especially when the CYP3A4 metabolism becomes saturated or down-regulated. However, the activity of CYP3A in TC7 microsomes is approximately 30-fold lower than that in human jejunal microsomes which in turn is approximately 6-fold lower than that in human hepatic microsomes (15). It is therefore possible that the Pgp-mediated efflux of terfenadine is less important in vivo than in the TC7 model. This is supported by recent studies of another Pgp-substrate, verapamil, which displays strongly polarized transport through Caco-2 cells (26). In these studies, it was concluded that Pgp has a limited influence on the permeability of the perfused human intestine to verapamil (10). These results indicate that while *in vitro* models such as Caco-2 cell monolayers can provide important qualitative information on the involvement of various transport and metabolism pathways in the absorption of orally administered drugs, they are not yet quantitatively predictive of the *in vivo* situation.

Both terfenadine and its metabolites were substrates for Pgp, with higher secretory than absorptive fluxes for all three compounds. It can therefore be speculated that Pgp contributes to the extensive accumulation of terfenadine metabolites in the faeces after oral administration. This result also points to a complex interplay between the transport and metabolism of terfenadine in the human intestinal epithelium. When CYP3A was inhibited by ketoconazole, HO-T was not formed and a dramatic increase in the absorptive flux of terfenadine was observed. This effect was higher than expected and was considered not entirely attributable to the enzymatic inhibition. A possible explanation was provided by the finding that ketoconazole also inhibited the secretory flux of the Pgp substrate digoxin in TC7 cells. Thus, the inhibition by ketoconazole of both CYP3A and Pgp most likely contributed to the enhanced absorptive flux of terfenadine. Whether Pgp also contributes to the important drug interaction between ketoconazole and terfenadine, which increases the plasma concentrations of terfenadine and leads to cardiotoxicity, remains to be seen (17). A recent human study on the effect of ketoconazole on intestinal verapamil permeability and metabolism suggests that the effect on

metabolism dominates (28). However, in that study the inhibitor to substrate ratio was much lower than in the present study. Thus, the results obtained in this and in other cell culture studies (27) as well as in transgenic mice (6,7) suggest that Pgp is a potentially important barrier to the bioavailability of orally administered drugs. In contrast, two recent human studies suggest the opposite (10, 28). However, in another human study, Pgp was shown to contribute to 17% of the variability of the oral clearance of cyclosporin (29). Additional human studies are required to resolve this issue.

In addition to Pgp and CYP3A, the very high lipophilicity of terfenadine (CLogP 6.09) as compared to its metabolites (CLogP 4.10 and 2.17 for HO-T and AC, respectively), may also have contributed to the reduced absorptive flux. Highly lipophilic compounds are generally very soluble in the cell membranes and are therefore slow to distribute to the water phase in the receiving compartment (30). In support of this hypothesis, a significant proportion of terfenadine were found associated to the cell membranes of the TC7 cells at the end of the transport experiments (15). We tentatively conclude that the absorptive flux of terfenadine was further reduced by a high association with the cell membranes of the TC7 monolayers.

In summary, the question of the relative importance of intestinal CYP3A and Pgp for the bioavailability of drugs remains open. The present study indicates that while cell culture models such as the TC7 cell monolayers can not yet resolve this issue, they provide qualitative data on the possible involvement of and interplay between these two barriers to absorption. Such information could be of profound importance in drug discovery programs. By identifying possible drug interactions with CYP3A and Pgp in cell culture models such as the TC7 monolayers, information regarding potential clinical drug interactions at the level of the intestinal epithelium can be obtained at an earlier stage than previously.

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