Transport Characteristics of Fexofenadine in the Caco-2 Cell Model

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Purpose. To investigate the membrane transport mechanisms of fexofenadine in the Caco-2 model.

Methods. Transport studies were performed in Caco-2 cell monolayers 21–25 days after seeding. The apparent permeability (P_{app}) of fexofenadine was determined in the concentration range 10–1000 μM in the basolateral-to-apical (b-a) and 50–1000 μM in the apical-to-basolateral (a-b) direction. The concentration-dependent effects of various inhibitors of P-glycoprotein (P-gp) (GF120918, ketoconazole, verapamil, erythromycin), multidrug resistant associated protein (MRP) (indomethacin, probenecid), and organic anion transporting polypeptide (OATP) (rifamycin SV) on the bidirectional transport of 150 μM fexofenadine were also examined.

Results. Fexofenadine displayed polarized transport, with the $P_{appb\text{-}a}$ being 28- to 85-fold higher than the $P_{app(a\text{-}b)}$. The $P_{app(a\text{-}b)}$ was independent of the concentration applied, whereas $P_{appb\text{-}a}$ decreased with increasing concentration (V_{max} = 5.21 nmol $cm^{-2}s^{-1}$ and K_M = 150 μM), suggesting saturation of an apical efflux transporter. All four P-gp inhibitors had a strong, concentration-dependent effect on the P_{app} of fexofenadine in both directions, with GF 120918 being the most specific among them. The IC_{50} of verapamil was 8.44 μM on the P-gp–mediated secretion of fexofenadine. The inhibitors of OATP or MRP appeared not to affect the $P_{app(a\text{-}b)}$ of fexofenadine in the Caco-2 model.

Conclusions. This study clearly indicates that P-gp was the main transport protein of fexofenadine in the Caco-2 model. Even though P-gp was completely inhibited, fexofenadine was predicted to have a low fraction dose absorbed in humans due to poor intestinal permeability, and low passive diffusion seems to be the major absorption mechanism.

KEY WORDS: absorption; Caco-2; fexofenadine; GF 120918; P-glycoprotein.

INTRODUCTION

Low and variable bioavailability is still considered to be one of the main reasons for terminating further development of oral pharmaceutical products (1,2). A successful development of drugs for oral drug delivery requires identification of the rate-limiting step(s) of the absorption and first-pass extraction process, such as dissolution, permeability, metabolism and biliary secretion. Studies examining the significance of membrane transport proteins in intestinal absorption and hepatic extraction are, therefore, important. The gut, and the intestinal epithelium in particular, has a high transport and metabolic capacity for a broad range of nutrients and xenobiotics. The carrier-mediated transport of drugs across the epithelium is complex. Several of the membrane transport proteins have overlapping substrate specificity and are located at either the apical or basolateral membrane, transporting drugs and metabolites in different directions. Several *in vivo* and *in vitro* techniques have to be applied to evaluate and understand the involvement of different membrane transporters in the overall epithelial transport.

Fexofenadine has been suggested to be a suitable *in vivo* probe to investigate the role of P-glycoprotein (P-gp, ABC B1) mediated membrane transport (3–5). Fexofenadine has interesting characteristics for that purpose such as a low human intestinal permeability and a minor degree of metabolism (6) and has been subjected to a number of drug-drug interactions (3,5,7,8). These clinical reports have suggested that intestinal P-gp was the main site for these interactions.

However, using a human *in vivo* jejunal perfusion technique (9) we have shown that concomitant administration of verapamil or ketoconazole did not increase the effective low jejunal permeability (P_{eff}) of fexofenadine (10,11). On the other hand, the systemic exposure of fexofenadine increased 4-fold when verapamil was co-administered, which suggests that the transporters in the first-pass liver extraction of fexofenadine were inhibited instead of the intestinal transport (11). The transporters involved may be sinusoidal members of organic anion transporting polypeptide (OATP, SLC21A) and/or canalicular P-gp (12,13). Previous *in vitro* data indicating that fexofenadine is a substrate for both transporters (12,13) supports this hypothesis for the drug-drug interaction. However, it is still unclear whether other drug membrane transporters, such as breast cancer resistance protein (BCRP, ABCG2) and multidrug resistant associated protein (MRP, ABCC) are involved in the membrane transport of fexofenadine, as they are known to be expressed in the intestine and the liver in humans (14,15).

The aim of the current study was to use the Caco-2 model to investigate further the transport and inhibition kinetics of the membrane transport mechanisms important for the *in vivo* pharmacokinetics of fexofenadine.

MATERIALS AND METHODS

Chemicals

Fexofenadine HCL was obtained from Hoechst Marion Roussel (Kansas City, MO, USA). The inhibitors used in the transport experiments (erythromycin, indomethacin, ketoconazole, probenecid, and rifamycin SV) were purchased from Sigma (St. Louis, MO, USA). GF 120918 and verapamil were kind gifts from GlaxoWellcome R&D (Stevenage, UK) and Knoll AG (Ludwigshafen, Germany), repectively. [^{14}C]Mannitol with specific radioactivity of 51.5 mCi/mmol was purchased from NEN-DuPont, (Life Science Products, Stockholm, Sweden).

Analytical Methods

A reversed phase-HPLC method with fluorescence detection was developed from the method of Coutant *et al.* (16) to enable fexofenadine to be quantified. The HPLC system consisted of a Shimadzu LC 10AD pump (Shimadzu, Kyoto, Japan), a Jasco FP-1520 intelligent flouroscence detector (Jasco, Kyoto, Japan) with excitation and emission wave-

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lengths at 230 and 280 nm, repectively, together with a CMA/200 automatic sample injection system (CMA/microdialysis AB, Solna, Sweden). The analytical column was a Nucleosil 100-5 C_{18} column, 150 × 4.6 mm i.d. Fexofenadine was eluted with a mobile phase that consisted of acetic acid buffer (pH 5.0, ionic strength 0.01): methanol:acetonitrile 42:35:23% (v/v), at a flow rate of 1 ml/min. The samples were injected directly or after dilution with HBSS with 1% DMSO. The injection volume was 50 μl.

Separate standard curves and quality controls were made for the intervals 518–32 nM (receiver) and 50–5.3 μM (donor). The limit of quantification for the samples from receiver and donor was set to 32 nM (CV 5.9%) and 5.3 μM (CV 6.1%), respectively. The CV of the interassay variability was below 20%. [^{14}C]Mannitol was analyzed using a Wallac WinSpectral 1414 liquid scintillation counter (Turku, Finland).

Cell Culture

The Caco-2 cells were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA) and used in the transport experiments between passage 28 to 30 and 44 to 45. The cells were cultured at 37°C, 90% relative humidity, and 5% CO_2 atmosphere and maintained in Dulbecco's modified Eagle's medium (DMEM) with L-glutamine containing 10% fetal bovine serum (FBS), 1% nonessential amino acids (MEM), and 100 U/ml and 100 μg/ml penicillin and streptomycin, respectively. All medium components used were purchased from Life Technologies AB (Paisely, UK), and the culturing medium was changed every other day.

After reaching 70–90% confluency, the cells were detached from the culture flask by addition of a trypsin-EDTA solution (0.25% trypsin and 0.2% EDTA) and seeded at a density of 88,500 cells/cm^2 on 12-mm (internal diameter) Transwell polycarbonate membrane inserts (0.4-μm pore size) and placed in Transwell 12-well clusters purchased from Costar (Life Technologies AB). The cells were used on days 21 to 25 post-seeding to obtain differentiated monolayers and a higher expression of transport proteins (17).

Transport Experiments

All the bidirectional transport experiments were conducted in Hank's balanced salt solution (HBSS) buffered to pH 7.4 with Hepes containing 1% dimethylsulfoxide (DMSO) at 37°C. The influence of a pH gradient on the permeability was avoided by using pH 7.4 on both the donor and the receiving side. Samples were taken from the receiving side after 90, 120, 150, and 180 min; the removed volume was replaced with a corresponding volume of HBSS solution at 37°C. Samples from the donor solution were taken at 0 and 180 min. Each experiment was performed in triplicate. The concentration-dependent permeability of fexofenadine was studied in the concentration range 10 to 1000 μM (10, 50, 100, 150, 200, 500, and 1000 μM) in both apical-to-basolateral (a-b) and basolateral-to-apical (b-a) directions. In the apical to basolateral direction at 10 μM, the concentration in the receiving chamber was below the detection limit.

The concentration-dependent effects of P-gp, MRP, and OATP inhibitors on the bidirectional transport of 150 μM fexofenadine were also examined. The inhibitors used were GF120918 (0.5, 2, 10 μM), verapamil (0.1, 1, 10, 25, 50, 100, 200 μM), erythromycin (10, 100, 200 μM), ketoconazole (10, 60, 100 μM), indomethacin (10, 100, 200 μM), probenecid (10, 100 μM) and rifamycin SV (1, 10, 100 μM). Prior to the inhibition experiments, the cells were preincubated with each inhibitor for 20 min at 450 rpm (BMG termostat, Labvision, Sweden) and 37°C. The inhibitors were added on both donor and receiving side to maintain a constant inhibiting concentration throughout the cell during the experiment. The solution for the volume compensation on the receiving side contained the relevant inhibitors at the indicated concentrations.

To assess the integrity of a monolayer prior to and after an experiment, the transepithelial electrical resistance (TEER) and the permeability of the paracellular marker [^{14}C]mannitol were measured. [^{14}C]Mannitol was used at a concentration of 0.1 mM and 1 μCi/ml. The cell monolayers were considered to be intact when the permeability of mannitol was less than 0.2×10^{-6} cm/s.

The apparent permeability coefficients (P_{app}) were calculated using Eq. 1,

$$P_{app} = \frac{dQ}{dt} \times \frac{1}{(A \times C_0)} \quad (1)$$

where dQ/dt is the linear appearance rate of mass in the receiver solution transported during sink conditions, A is the surface area of the membrane, and C_0 is the initial donor concentration. The efflux ratio (i.e., the net efflux of fexofenadine) was determined by calculating the ratio of P_{app} in the secretory (b-a) divided by the absorptive (a-b) direction according to Eq. 2.

$$ER = \frac{P_{app(b\text{-}a)}}{P_{app(a\text{-}b)}} \quad (2)$$

Determination of the Apparent Maximum Velocity (V_{max}) and Michaelis-Menten Constant (K_M) of Fexofenadine and the IC_{50} of Verapamil

The concentration-dependent membrane transport rate of fexofenadine in both directions was studied in the concentration range 10–1000 μM. The passive membrane transport rate (excluding the influence of P-gp–mediated transport) of fexofenadine was estimated by conducting a transport experiment in the presence of the specific P-gp inhibitor GF 120918 (2 μM). The carrier-mediated membrane transport rate was the remainder of the transport rate in the presence of GF120918 subtracted from the total transport rate (18). The IC_{50} of verapamil on fexofenadine transport in the secretory direction was determined by using the percentage inhibited for each verapamil concentration. The percentage inhibited was calculated by dividing the $P_{app(b\text{-}a)}$ by the $P_{app(b\text{-}a)}$ for the control (150 μM fexofenadine). Michaelis-Menten parameters and IC_{50} were then determined using nonlinear regression according to the Hill equation with Graphpad Prism 3.02 (Graphpad Software Inc, USA).

Statistical Analysis

All values are presented as a mean ± one standard deviation (SD) throughout the paper. The statistical evaluation of the concentration dependency and the impact of the different inhibitors was performed with one-way ANOVA followed by Dunnet's and Bonferroni's multiple comparison

test, respectively. A probability of less than 0.05 ($p < 0.05$) was considered to be statistically significant.

RESULTS

The absorptive permeability ($P_{app(a-b)}$) of fexofenadine was low and independent of the apical concentration throughout the range investigated, 10–1000 μM (Table I and Fig. 1). On the other hand, the permeability in the secretory direction ($P_{app(b-a)}$) decreased at higher basolateral concentrations of fexofenadine, and, accordingly, the efflux ratio was reduced from 85 at 50 μM to 28 at 1000 μM (Table I). The membrane transport kinetics of fexofenadine in the secretory direction were described with the apparent V_{max} and K_M, which were 5.21 nmol cm^{-2} s^{-1} and 150 μM, respectively (Fig. 1).

Several different inhibitors (namely, GF 120918, ketoconazole, verapamil, erythromycin, rifamycin SV, indomethacin, and probenecid) were used to investigate the membrane transport protein(s) involved in the cellular transport of fexofenadine. The determined permeability across the Caco-2 monolayer, presented in Table II and Fig. 2, was compared against a control for fexofenadine (150 μM), which had a P_{app} of 0.31 ± 0.03 and $8.24 \pm 0.31 \times 10^{-6}$ cm s^{-1} in the absorptive and secretory directions, respectively. GF 120918, a specific inhibitor of P-gp, already increased the absorption and decreased the secretion at a concentration of 0.5 μM, which resulted in a efflux ratio of 1.1 (Table II). An additional increase in the concentration of GF 120918 did not affect the permeability in any direction, which was in accordance with complete inhibition of the P-gp–mediated membrane transport process. The cumulative percentage absorbed and transported across the membrane in the absorptive direction was less than 1% of the donor concentration when no efflux process was involved (i.e., when GF120918 at 2 μM was used) (Fig. 3). The passive P_{app} for fexofenadine used for calculations of membrane kinetics was 1.03 ± 0.07 and $1.06 \pm 0.07 \times 10^{-6}$ cm s^{-1} in the absorptive and secretory directions, respectively (Table II; 2 μM GF 120918).

The P-gp inhibitors ketoconazole and verapamil displayed a concentration-dependent inhibition of fexofenadine transport in both the absorptive and the secretory directions. The efflux ratio approached one with increasing inhibitor concentration (Table II). The $P_{app(a-b)}$ increased to 1.47 ± 0.13 and $1.84 \pm 0.09 \times 10^{-6}$ cm s^{-1} for verapamil and ketoconazole, respectively, at the highest inhibitor concentration (Table II).

Table I. The Concentration-Dependent Apparent Permeability (P_{app}) of Fexofenadine (10–1000 μM) in the Absorptive (a-b) and Secretory (b-a) Direction, and the Efflux Ratio (ER) in the Caco-2 Model

	P_{app} ($\times 10^{-6}$ cm s^{-1})				
Donor (μM)	a-b	p	b-a	p	ER
10			14.22 ± 0.53		—
50	0.17 ± 0.08		14.02 ± 1.71	>0.05	85
100	0.23 ± 0.03	>0.05	14.82 ± 0.95	>0.05	66
200	0.27 ± 0.04	<0.05	10.19 ± 0.23	<0.001	37
500	0.22 ± 0.03	>0.05	8.78 ± 1.04	<0.001	40
1000	0.19 ± 0.01	>0.05	5.43 ± 0.42	<0.001	28

One-way ANOVA with Dunnet's multiple comparison test [10 μM (b-a) and 50 μM (a-b) as control].

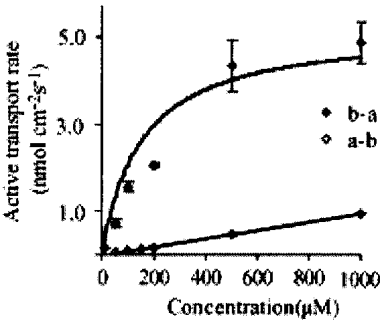

Fig. 1. The concentration dependency of the active transport in the secretory (b-a) direction of fexofenadine across Caco-2 monolayers. The passive diffusion flux rates were determined in the presence of GF120918 (2 μM), which totally inhibits the active transport of fexofenadine. The active transport rates were determined by subtracting the passive diffusion flux rates from the total flux rates in accordance with Gao *et al.* (18). Apparent Michaelis-Menten parameters were estimated using Graphpad Prism 3.02 as described under "Materials and Methods." Data points are means ± SD (n = 3).

This was higher than the established passive permeability with GF 120918, but the increase was not associated with any effect on the mannitol permeability. These $P_{app(a-b)}$ values, however, still predict a low permeability and an incomplete absorption in humans (19). The inhibition with verapamil was more extensively investigated, and the IC_{50} was determined to be 8.44 μM (Fig. 4). Erythromycin affected the permeability for fexofenadine in the absorptive direction in the same manner as ketoconazole and verapamil (Table II), but to a lesser extent.

Rifamycin SV did not significantly alter the permeability of fexofenadine in the absorptive direction, but interestingly a decrease in $P_{app(b-a)}$ was observed at 100 μM of the inhibitor (Fig. 2, Table II). The MRP inhibitor indomethacin did not affect the permeability of fexofenadine in any direction. On the other hand, probenecid, an inhibitor of anion (MRP-related) membrane transport, decreased the $P_{app(a-b)}$ and increased the $P_{app(b-a)}$ (Fig. 2; Table II).

DISCUSSION

In two previous *in vivo* jejunal perfusion studies, we investigated the transport interactions of fexofenadine and two inhibitors, ketoconazole and verapamil, at a mechanistic level in the human intestine (10,11). This clinical technique gives a possibility to determine the *in vivo* transport rate of a drug directly and to examine the maximal risk of a drug-drug interaction in the intestine. It is important to compare these *in vivo* data with the corresponding data from the *in vitro* cell monolayer, as this model may be used to predict drug-drug interaction at a transport level. The aim of the current *in vitro* study was to elucidate the membrane transport processes that were crucial for the absorption of fexofenadine by examining the effect of various transport protein inhibitors as well as to estimate the role of passive diffusion in the overall membrane transport and to characterize the transport and inhibition kinetics using Caco-2 monolayers.

The current results clearly indicate that P-gp was highly involved in the active membrane transport of fexofenadine in this model. The permeability was affected by the P-gp inhibitors in both the absorptive and secretory directions. GF

Table II. The Apparent Permeability (P_{app}) of Fexofenadine Applied in Combination with Various Inhibitors of Transport Proteins, P-gp, BCRP, OATP, MRP, in the Absorptive (a-b) and Secretory (b-a) Direction Across Caco-2 Monolayers

			a-b		b-a		
Inhibitor	Donor (μM)	Inhibited transporter	P_{app} ($\times 10^{-6}$ cm s^{-1})	Change (%)	P_{app} ($\times 10^{-6}$ cm s^{-1})	Change (%)	ER
Control	150	—	0.31 ± 0.03		8.24 ± 0.31		26.2
GF 120918	0.5	P-gp, BCRP	0.95 ± 0.10***	201 ± 10	1.04 ± 0.06***	−87 ± 6	1.1
	2		1.03 ± 0.07***	227 ± 7	1.06 ± 0.08***	−87 ± 8	1.0
	10		1.03 ± 0.01***	229 ± 1	1.12 ± 0.10***	−86 ± 9	1.1
Ketoconazole	10	P-gp, OATP	0.99 ± 1.10***	214 ± 10	4.45 ± 0.38***	−46 ± 9	4.5
	60		1.49 ± 0.12***	374 ± 8	3.32 ± 0.18***	−60 ± 5	2.2
	100		1.84 ± 0.09***	484 ± 5	3.07 ± 0.12***	−63 ± 4	1.7
Verapamil	10	P-gp, OATP	0.58 ± 0.04*	86 ± 7	7.63 ± 0.64 n.s.	−7.4 ± 8	13.1
	100		1.08 ± 0.05***	243 ± 4	2.92 ± 0.24***	−65 ± 8	2.7
	200		1.47 ± 0.13***	368 ± 9	2.13 ± 0.30***	−74 ± 14	1.4
Erythromycin	10	P-gp, OATP	0.20 ± 0.04*	−36 ± 22	13.06 ± 0.55**	58 ± 4	65.1
	100		0.41 ± 0.02*	29 ± 4	11.41 ± 1.21*	38 ± 11	28.1
	200		0.77 ± 0.03***	146 ± 4	8.64 ± 1.27 n.s.	4.8 ± 15	11.2
Rifamycin SV	1	OATP, P-gp	0.25 ± 0.05 n.s.	−21 ± 21	8.58 ± 0.38 n.s.	4.1 ± 4	34.7
	10		0.21 ± 0.02 n.s.	−34 ± 8	8.47 ± 0.29 n.s.	2.8 ± 3	40.6
	100		0.36 ± 0.05 n.s.	14 ± 15	5.54 ± 0.32***	−33 ± 6	15.5
Indomethacin	10	MRP, not P-gp	0.24 ± 0.04 n.s.	−22 ± 18	7.54 ± 0.79 n.s.	−8.5 ± 10	30.9
	100		0.26 ± 0.03 n.s.	−18 ± 13	8.12 ± 0.81 n.s.	−1.5 ± 10	31.6
	200		0.31 ± 0.01 n.s.	0 ± 3	7.17 ± 0.85 n.s.	−13 ± 12	22.8
Probenecid	10	MRP, not P-gp	0.23 ± 0.01*	−28 ± 4	14.78 ± 0.64***	79 ± 4	65.0
	100		0.16 ± 0.04*	−49 ± 24	14.28 ± 0.16***	73 ± 1	89.1

One-way ANOVA and Bonferroni's multiple comparison test compared against the control.
*** $p < 0.001$, **$p < 0.01$, *$p < 0.05$, n.s. $p > 0.05$.

120918 was the most potent and specific inhibitor of the four P-gp inhibitors used. A previous study had revealed that GF 120918 did not affect MRP1 and 2 (20), but it has an inhibitory effect on BCRP at concentrations at and above 10 μM (21). The efflux ratio of fexofenadine was 1 at 0.5 μM of GF120918, which indicates that P-gp is the only efflux transport protein involved in the active membrane transport of fexofenadine in the Caco-2 model. No additional changes in permeability or in the efflux ratio were observed when the concentration of GF 120918 was increased. This suggests that BCRP was not involved in the membrane transport of fexofenadine since the current Caco-2 model has an intermediate expression of BCRP (Ungell A-L., personal communication).

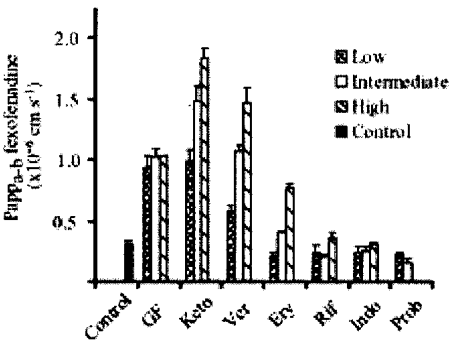

Fig. 2. The apparent permeability (P_{app}) of fexofenadine (150 μM) with various transport inhibitors in the absorptive direction (a-b) across Caco-2 monolayers. The inhibitors used were GF120918 (0.5, 2, 10 μM), verapamil (10, 100, 200 μM), erythromycin (10, 100, 200 μM), ketoconazole (10, 60, 100 μM), indomethacin (10, 100, 200 μM), probenecid (10, 100 μM) and rifamycin SV(1, 10, 100 μM).

MRP was not believed to be involved in the membrane transport of fexofenadine, as the inhibitor indomethacin had no effect on the cellular efflux. Surprisingly, probenecid led to a decrease in the permeability in the absorptive direction and an increase in the secretory direction, but these observations were not considered to be the result of a direct carrier-mediated effect, as indomethacin had no effect on the permeability and because the specific inhibitor GF 120918 completely inhibited the cellular efflux. In addition, previous reports have shown that probenecid did not affect the cellular transport of fexofenadine (13). Although both probenecid and indomethacin are thought to be rather unspecific as transport inhibitors, it has been reported that they do not affect the P-gp mediated activity (22,23), which was also supported by the current study. It may be speculated that the difference in effect of probenecid in the current study and that by Perloff

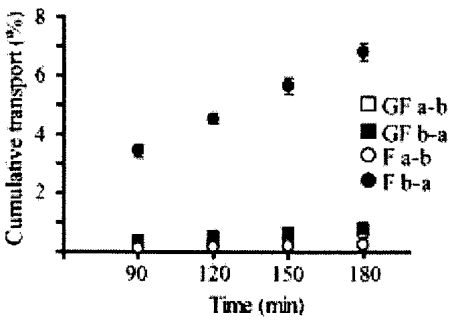

Fig. 3. The cumulative transport of fexofenadine (150 μM) alone or in the presence of the specific P-gp inhibitor GF 120918 (2 μM), in the absorptive (a-b) and secretory (b-a) direction across Caco-2 monolayers.

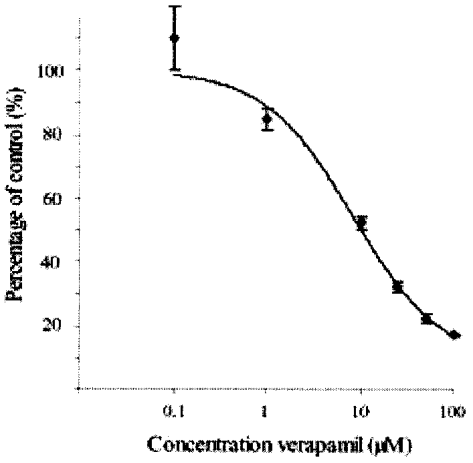

Fig. 4. Inhibition of fexofenadine transport in the secretory (b-a) direction by verapamil (0.1–100 μM) across Caco-2 monolayers. Data presented as a percentage of the uninhibited control. Each data point represents the mean ± SD (n = 3). The IC_{50} value for verapamil on fexofenadine transport was estimated by a nonlinear regression using Graphpad Prism 3.02.

et al. might be because probenecid inhibits an uptake membrane protein, such as OATP, which is not present in their cell model (13). It is well-known that the expression of these efflux transporter exhibit significant inter- and intra-laboratory variability. However, the most likely explanation is that probenecid may cause various and unexplained effects due to its inhibition of intracellular metabolism related to oxidative mitochondrial metabolism (24). It supports previous studies that probenecid should be used with cautios as a pharmacological tool in cellular research (24).

OATP has been shown to transport fexofenadine in a concentration-dependent manner in a transfected cell line (12). However, even though certain members of OATP (namely, OATP-B, OATP-D, OATP-E) have been reported to be expressed in the intestine (25,26), the expression of functional OATP in Caco-2 is uncertain even if high gene expression of OATP-B has been found in the current Caco-2 model (Ungell A-L., personal communication). In the current study, no OATP-mediated uptake could be distinguished from the efflux transport. The absent OATP activity was confirmed with rifamycin SV, an inhibitor of several members of OATP (27). The only effect of the inhibitor was a decrease of fexofenadine transport in the secretory direction at a concentration of 100 μM. However, this observation may be caused by an inhibition of P-gp, as rifamycin SV has structural similarities to rifampin, which has been shown to have an inhibitory effect on P-gp in humans (3). This is also supported by that rifamycin SV has been shown to accumulate vinblastine in rat hepatocytes at concentrations of 100 μM and above (28). Another possible explanation to the absent OATP effect may be that the activity and specificity of any present OATP is lower at pH 7.4. A recent report have shown that the activity was higher and specificity was lower at acidic pH for OATP-B (26), which is apically located. Interestingly, Cvetkovic *et al.* (12) have also shown that verapamil, ketoconazole, and erythromycin inhibited the OATP transport of fexofenadine using concentrations similar to those in this study. Nevertheless, these effects were not observed or could not be distinguished from the P-gp transport. The involvement of other transporters has not been extensively investigated, but the rat organic cation transporter 1(rOCT1) and the human sodium taurocholate cotransporting polypeptide (NTCP) do not seem to transport fexofenadine (12,13,29)

Fexofenadine is an example of an essentially nonmetabolized substance (6) with a low human intestinal permeability, which has altered pharmacokinetics when it was administered with known P-gp inhibitors and inducers (3,7,8,10,11). In the current and other studies (12,13), the *in vitro* inhibition data support the hypothesis of P-gp being a factor for the absorption and bioavailability of fexofenadine, so it was interesting to note that the systemic availability of fexofenadine in humans was linear for oral doses from 10 to 800 mg (30). The absence of a dose-dependent effect on the *in vivo* absorption and first-pass extraction was in accordance with the concentration-independent $P_{app(a-b)}$ found in the current *in vitro* study. On the other hand, the $P_{app(b-a)}$ was highly concentration dependent, indicating saturation of a P-gp–mediated secretion of fexofenadine. This asymmetric difference in the concentration-dependent permeability suggests that the P-gp binding site(s) may be more accessible from the basolateral side. This may be explained by apical membranes displaying a lower inherent passive permeability than basolateral membranes. The rate-determining factor for the low permeability is believed to be attributable to the structure of the exofacial leaflet of the apical membrane (31). This should be taken into consideration as the binding site(s) of P-gp may be located in the inner membrane leaflet toward the cytoplasm and hence was exposed to a higher intracellular concentration when fexofenadine was applied on the basolateral side. As the molecular weight of fexofenadine is 538 g mol^{-1} and as the P-gp inhibitors increased the $P_{app(a-b)}$, it may be concluded that fexofenadine is transported transcellularly rather than by the paracellular route (32).

In the evaluation of the role of efflux proteins in the overall transport of substrates, it is also important to consider the contribution from passive diffusion of the compound. Even if a compound is efficiently transported by P-gp, it can still have a high fraction dose absorbed in humans (33). The reasons for the high dose absorbed could be several: the compound has a high passive permeability across the cell membrane, and the fraction of the drug dose that the efflux transporter pumps back out into the lumen may be reabsorbed along the small and large intestine; the expression of the transporter may be different along the intestine; or it can be saturated by the dose of the compound given. Therefore, it is generally believed that P-gp and other efflux transporters would have the strongest effect on the fraction dose absorbed and hence the bioavailability of a low permeability drug such as fexofenadine. According to a classification recently proposed by Troutman and Thakker (34), the absorptive quotient of fexofenadine was calculated to be 0.7 (class I), meaning that P-gp attenuates the passive permeability during absorption by 70% in the Caco-2 model. However the low permeability found both *in vitro* and *in vivo* does not have to be caused by extensive intestinal efflux, despite the fact that the compound is a substrate for P-gp. As shown in the current study, when the efflux was completely inhibited by GF 120918, the $P_{app(a-b)}$ was $1.03 \pm 0.07 \times 10^{-6}$ cm/s. The permeability of fexofenadine increased by 229%, but it was still a low permeability compound with less then 1% of the total amount transported in the absorptive direction. The physico-

chemical properties of fexofenadine (a zwitterion), such as the degree of ionization, molecular weight, and the polar surface area (124 $Å^2$), predict that a low passive permeability and a low fraction dose will be absorbed in humans (35,36). This was also confirmed in healthy volunteers as the human intestinal effective permeability (P_{eff}) was determined to be low (10,11). Altogether, these data demonstrate that there is still poor understanding of the significance of efflux transporters in the human intestinal mucosa and how, or if, they limit drug absorption

In conclusion, the result from this study clearly indicates that P-gp is the dominating transport protein in the Caco-2 model, but not the major reason for the incomplete intestinal absorption. The permeability for fexofenadine across the model can be modified to some extent by P-gp inhibitors, but the permeability remained low when the transport proteins were completely inhibited, and the transport in the Caco-2 model was predominantly governed by passive permeability and physiochemical properties.

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