British Journal of Pharmacology (1997) 121, 1567–1578 © 1997 Stockton Press All rights reserved 0007–1188/97 $12.00

Fluoroquinolone (ciprofloxacin) secretion by human intestinal epithelial (Caco-2) cells

M.E. Cavet, *M. West & [1]N.L. Simmons

Gastrointestinal Drug Delivery Research Centre, Department of Physiological Sciences, University of Newcastle upon Tyne, Medical School, Newcastle upon Tyne, NE2 4HH and *Glaxo Medicines Research Centre, Grunnelswood Road, Stevenage, Hertfordshire, SG1 2NY

1 Human intestinal epithelial Caco-2 cells were used to investigate the mechanistic basis of transepithelial secretion of the fluoroquinolone antibiotic ciprofloxacin.

2 Net secretion and cellular uptake of ciprofloxacin (at 0.1 mM) were not subject to competitive inhibition by sulphate, thiosulphate, oxalate, succinate and *para*-amino hippurate, probenecid (10 mM), taurocholate (100 μM) or bromosulphophthalein (100 μM). Similarly tetraethylammonium and N-′methylnicotinamide (10 mM) were without effect.

3 Net secretion of ciprofloxacin was inhibited by the organic exchange inhibitor 4,4′-diisothiocyanostilbene-2-2′-disulphonic acid (DIDS, 400 μM).

4 Net secretion of ciprofloxacin was partially inhibited by 100 μM verapamil, whilst net secretion of the P-glycoprotein substrate vinblastine was totally abolished under these conditions. Ciprofloxacin secretion was unaltered after preincubation of cells with two anti-P-glycoprotein antibodies (UIC2 and MRK16), which both significantly reduced secretory vinblastine flux (measured in the same cell batch). Ciprofloxacin (3 mM) failed to inhibit vinblastine net secretion in Caco-2 epithelia, and was not itself secreted by the P-glycoprotein expressing and vinblastine secreting dog kidney cell line, MDCK.

5 Net secretion and cellular uptake of ciprofloxacin (at 0.1 mM) were not subject to alterations of either cytosolic or medium pH, or dependent on the presence of medium Na^+, Cl^- or K^+ in the bathing media.

6 The substrate specificity of the ciprofloxacin secretory transport in Caco-2 epithelia is distinct from both the renal organic anion and cation transport. A role for P-glycoprotein in ciprofloxacin secretion may also be excluded. A novel transport mechanism, sensitive to both DIDS and verapamil mediates secretion of ciprofloxacin by human intestinal Caco-2 epithelia.

Keywords: Ciprofloxacin; Caco-2 cells; epithelia; intestinal secretion; P-glycoprotein

Introduction

The major route of elimination of the fluoroquinolone antibiotic ciprofloxacin is via the kidneys (Jaehde *et al*., 1989; Ramon *et al*., 1994; Rohwedder *et al*., 1990; Rubinstein *et al*., 1994; 1995; Sorgel *et al*., 1989; 1991). However, there is extensive evidence for a significant trans-intestinal elimination of ciprofloxacin (Sorgel *et al*., 1989; 1991; Jaehde *et al*., 1989; Rohwedder *et al*., 1990). Since biliary elimination accounts for less then 1% of an intravenous dose and only a minor component of ciprofloxacin in faeces is metabolites (Parry *et al*., 1988; Rohwedder *et al*., 1990; Ramon *et al*., 1994; Rubenstein *et al*., 1994, 1995), this suggests the existence of a specific intestinal secretory mechanism for ciprofloxan.

Active, saturable, intestinal secretion of ciprofloxacin has been demonstrated *in vitro* with the human intestinal cell line Caco-2 (Griffiths *et al*., 1993). Secretory transport of ciprofloxacin involves sequential transport across two separate membranes (the apical and basolateral) arranged in series; there is evidence for distinct membrane transporters for fluoroquinolones at the apical and basolateral membranes (Griffiths *et al*., 1994). However, the identity and pharmacological characteristics of the transporter(s) involved in the transport at both basolateral and apical membranes is unknown and requires further characterization.

Ciprofloxacin is zwitterionic at physiological pH 7.4 ($pK_{a1} = 6.5$, $pK_{a2} = 8.5$ the isoelectric point = 7.4) due to a negatively charged carboxyl group and a positively charged nitrogen of the piperazine ring (Sorgel & Kinzig, 1993a). For renal secretion it appears that ciprofloxacin and other fluoroquinolones are transported primarily via the renal organic anion, though it is also known that ciprofloxacin may interact with the basolateral renal organic cation transporter with moderate affinity (Sorgel & Kinzig, 1993b; Ullrich *et al*., 1993a). Ciprofloxacin may thus act as a 'bisubstrate' for both organic anionic and cation transports. Renal organic anion transport is coupled to the movement of Na^+ at both the basolateral and apical membranes (Ullrich & Rumrich, 1988), whilst the uptake of the organic anion bromosulphophthalein is coupled to Cl^- in the liver (Wolkoff *et al*., 1987; Jaquemin *et al*., 1991). Three distinct renal basolateral organic anion transport systems may be distinguished on the basis of substrate and inhibitor specificity: (a) the sulphate transport system, (b) the dicarboxylate transporter and (c) the para amino hippurate (PAH) transporter (Ullrich & Rumrich, 1988). The relationship between transporters mediating renal and intestinal fluoroquinolone secretion has not been examined.

Intestinal secretion of both organic anions and cations has been demonstrated in guinea-pig isolated intestinal mucosa (Lauterbach, 1977a,b; 1983; Turnheim & Lauterbach, 1972; 1977; 1980). An H^+/guanidine exchanger has been demonstrated in brush border membrane vesicles from rabbit (Miyamoto *et al*., 1988). The secretory transport of anionic β-lactam antibiotics across rat intestinal segments *in vitro* can be inhibited by substrates for both renal organic anion and cation transporters (Saitoh *et al*., 1996). The intestine is also capable of the transport of lipophilic cationic drugs via the adenosine 5′-triphosphate (ATP)-driven efflux pump P-glycoprotein, mediating transepithelial secretion of substrates such as vinblastine and digoxin (Hunter *et al*., 1993a,c; Cavet *et al*., 1996; Mayer *et al*., 1996). It has recently been suggested that P-glycoprotein is involved in the transepithelial secretion of ofloxacin and ciprofloxacin in the rat (Rabbaa *et al*., 1995). ATP-dependent extrusion of glutathione conjugates has also been documented in Caco-2 intestinal epithelial cells; 1-chloro-2,4-dinitrobenzene (CDNB) is a substrate for glutathione-S-

[1] Author for correspondence.

transferase, dinitrophenyl-S-glutathione (DNP-SG) then being subject to ATP dependent secretion in Caco-2 epithelia (Elferink *et al*., 1993). It is now apparent that ATP-dependent extrusion of glutathione conjugates results from expression of the multidrug resistance-associated protein (MRP) (Evers *et al*., 1996; Flens *et al*., 1996). A Na^+ – independent transporter of bile acids has recently been cloned from rat liver (Jacquemin *et al*., 1994) and a cDNA probe has been found to hybridize with mRNA from rat proximal colon.

It is evident that a number of primary active (ATP-dependent) or secondary active transport systems (coupled to H^+, Na^+, and or Cl^- gradients) may be responsible for transepithelial ciprofloxacin secretion in human Caco-2 intestinal epithelia. The purpose of this work has been to determine the extent and role of identified transporters to intestinal ciprofloxacin secretion, whilst allowing a comparison of intestinal secretion of ciprofloxacin by Caco-2 epithelia with renal organic anion and cation transport. The data have been used to define a cellular model for ciprofloxacin secretion by Caco-2 cell monolayer.

Methods

Cell culture

Caco-2 cells were obtained from Dr I. Hussan (Ciba-Geigy Pharmaceuticals, Horsham, Sussex, U.K.) and used between passage number 95 – 114. Two distinct MDCK strains, I (76 – 78 serial passages) and strain II (120 – 122 serial passages), were used (Hunter *et al*, 1993b). Caco-2 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing glucose (4.5 g l^{-1}) and supplemented with non-essential amino acids (1%), L-glutamine (2 mM), foetal calf serum (10%) and gentamycin (60 μg ml^{-1}). MDCK cells were cultured in Eagle's Minimal Essential Medium (EMEM), supplemented with non-essential amino acids (2%), L-glutamine (4 mM), foetal calf serum (10%) and kanamycin (100 μg ml^{-1}). Cell monolayers were prepared by seeding at high density ($4.4 - 5.0 \times 10^5$ cells cm^{-2}) onto tissue culture inserts (Transwell 3412, 24 mm diameter, 0.4 μm pore size uncoated polycarbonate filters (Costar)). Cell monolayers were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air. Confluence of Caco-2 and strain I MDCK epithelia was estimated by microscopy and determination of transepithelial electrical resistance (R_T) with a WPI Evometer fitted with 'chopstick' electrodes (World Precision Instruments, Stevenage, Hertfordshire), measured at 37°C in Krebs buffer (Hunter *et al*., 1993a,c), typically 200 – 300 Ω cm^2 for Caco-2 cells and 1000 – 2000 Ω cm^2 for MDCKI cells. MDCK II epithelia have a low R_T but a cation-selective paracellular pathway so that a choline: Na bi-ionic potential difference (p.d.) across the monolayer was measured (Barker & Simmons, 1981) by the Evometer as above, after isosmotic replacement of NaCl in the basolateral bathing Krebs buffer with choline chloride for 5 min (typical p.d. exceeded 30 mV (basolateral solution electropositive)).

Transepithelial transport experiments

Experimental solutions The modified krebs buffer contained (mmol l^{-1}): NaCl 137, KCl 5.4, $CaCl_2$ 2.8, $MgSO_4$ 1.0, NaH_2PO_4 0.3, KH_2PO_4 0.3, glucose 10 and HEPES/Tris 10 (pH 7.4, 37°C). A Na^+-free medium was made by replacement of NaCl by choline Cl and NaH_2PO_4 by the K salt. A K^+-free medium was made by omitting K salts. A Cl^--free medium was obtained by replacement of NaCl, KCl and $CaCl_2$ by their respective gluconate salts. Inhibitory salts e.g. sulphate, thiosulphate oxalate, succinate and *para*-amino hippurate were added as Na salts directly into Krebs.

Uptake and transport experiments Uptake and transport experiments with ciprofloxacin were performed 14 – 21 days after seeding and 18 – 24 h after feeding for Caco-2 epithelia. Ciprofloxacin accumulating in the apical compartment after transport through Caco-2 cells was confirmed as native drug by high performance liquid chromatography (h.p.l.c.) and mass spectrometry (Cavet, 1996). Vinblastine is known to be secreted by the renal tubular MDCK I and MDCK II cells by a P-glycoprotein-dependent mechanism (Hunter *et al*., 1993b), thus epithelial monolayers of MDCK cells were used to test whether P-glycoprotein contributes to ciprofloxacin secretion, by comparing ciprofloxacin transport in MDCK epithelia to Caco-2 epithelia. MDCK epithelia were used 3 – 5 days after seeding. Transepithelial flux measurements were performed at steady state as described previously in a modified Krebs solution (Cavet *et al*., 1996). The experimental composition of the buffers in the apical and basal chambers were identical except where stated otherwise. Radiolabelled [^{14}C]-ciprofloxacin and [^{3}H]-mannitol (0.1 μCi ml^{-1}, 5 – 10 μM for ciprofloxacin and 3 – 5 μM for mannitol) were added to either the apical or basolateral chamber and in each case an equivalent concentration of unlabelled substrate was present in the contra lateral chamber. For the majority of experiments unlabelled ciprofloxacin was present in excess of radiolabel. The total unlabelled ciprofloxacin concentration is indicated in figure and table legends and was equal in both apical and basal bathing solutions. Whether other drugs were present equal concentrations were present in both the apical and basolateral bathing solutions except where indicated. Fluxes in the absorptive (apical-to-basal, $J_{a\text{-}b}$) and secretory (basal-to-apical, $J_{b\text{-}a}$) directions were determined for 1 h (after a 20 min preincubation to establish a state of linear flux) on adjacent paired cell monolayers and are expressed as nmol cm^{-2} h^{-1} or pmol cm^2 h^{-1}. The linearity of ciprofloxacin flux in three consecutive flux periods at 1, 2 and 3 h was confirmed in control experiments, cumulative $J_{b\text{-}a}$ at 10 μM ciprofloxacin being 0.64 ± 0.03, 1.28 ± 0.04 and 1.76 ± 0.05 nmol cm^{-2}, respectively ($n = 3$). The passive (paracellular) route across the epithelium was estimated by concurrent mannitol flux determinations. Mannitol flux into the contralateral chamber was typically 2% at the end of the incubation period. Flux values of $>5\%$ led to rejection of the monolayer and associated ciprofloxacin flux determination.

At the end of the incubation period cell monolayer were washed by sequential transfer through 4 beakers containing 500 ml volumes of Krebs buffer (pH 7.4) at 4°C to remove any loosely-associated radiolabel, and removed from the insert. Cell monolayer-associated radiolabel was determined by scintillation counting. Cellular uptake of ciprofloxacin is expressed as μM or as a cell/medium (C/M) ratio. Cell height (h) (mean of 20 determinations from 3 layers) was determined directly by x-z confocal imaging of intact epithelial layers and this value was used in the determination of intracellular volume (πr^2 h = 8.1 μl, where r is the radius of the filter insert) (Thwaites *et al*., 1993a,b).

Where both transepithelial bidirectional fluxes were measured, epithelial cell monolayers were paired during experimental design for determination of $J_{a\text{-}b}$ or $J_{b\text{-}a}$ and net transepithelial flux determined ($J_{net} = J_{a\text{-}b} - J_{b\text{-}a}$). In addition, where cellular uptake was determined across the apical and basolateral cell surfaces, it is possible to calculate unidirectional fluxes across the apical and basolateral border (Naftalin & Curran, 1974) from the following equations:

$$J_{a-c} = J_{b-a}R + J_{a-b}$$

$$J_{c-a} = J_{b-a}\ (1 + R)$$

$$J_{c-b} = J_{a-b}\ (1 + 1/R)$$

$$J_{b-c} = J_{b-a} + J_{a-b}/R$$

where J denotes the respective fluxes; subscripts a, b and c denote apical, basolateral and cell compartments, R is the ratio of cellular uptake of the substrate from the apical media divided by that from basal media. Permeabilities, $P_{a\text{-}c}$ etc were calculated $= J_{a\text{-}c}/C_a$ where C_a is the concentration of ciprofloxacin in the ipsilateral (originating) compartment.

Determinations of the transepithelial transport of vinblastine sulphate (10 nM – 100 μM vinblastine sulphate with [^{3}H]-vinblastine sulphate as tracer) were made identically to ciprofloxacin, using [^{14}C]-mannitol as the paracellular marker.

Experiments manipulating medium and cytosolic pH

In order to test whether H^+/symport of H^+/antiport is involved in ciprofloxacin secretion, the effects of changes in intracellular and extracellular pH were investigated. Also, changes in pH will modify the concentration of ciprofloxacin in zwitterionic and acidic and basic form thus determining whether there is preferential transport of a particular species. Medium pH was varied in the range 6.0 to 7.4 by use of a 10 mM MES buffer adjusted to the required pH with Tris base, or to 8.5 (10 mM Tris adjusted with MES). The effect of intracellular acidification was investigated by prepulsing with 30 mM NH_4Cl for 15 min and then incubating in NH_4Cl-free media. This lowers the intracellular pH (pH_i) by approximately 1 pH unit and causes a marked inhibition in H^+/peptide and amino acid transport at the apical membrane of Caco-2 cells (Thwaites & Simmons, 1996). In Na^+ free conditions pH_i recovery is prevented (Thwaites *et al*., 1994).

Experiments with anti-P glycoprotein antibodies

The apical surfaces of Caco-2 cell layers were exposed to monoclonal antibodies UIC2 or MRK16 at 25 mg ml^{-1} in Krebs for 2 h before the flux determination. Control cell layers were incubated with a non-specific antibody of the same species and class (mouse IgG1 or IgG2a respectively). Antibodies were also present during the flux determination.

Materials

[^{14}C]-ciprofloxacin (specific activity 258.26 μCi mg^{-1}) and unlabelled ciprofloxacin were generous gifts from Bayer (Wuppertal, Germany). [^{3}H]-mannitol (specific activity 30 Ci mmol^{-1}) and [^{3}H]-vinblastine sulphate (specific activity 16 Ci mmol^{-1}) were from Amersham (Little Chalfont, Buckinghamshire, U.K.). [^{14}C]-mannitol (specific activity 50 Ci mmol^{-1}) was from New England Nuclear (Stevenage, Hertfordshire). Cell culture media, supplements and plastic were supplied by Life Technologies (Paisley, Strathclyde). Monoclonal anti P-glycoprotein MRK16 antibody was purchased from Kamiya Biomedical Company (Thousand Oaks, CA, U.S.A.). Monoclonal anti P-glycoprotein UIC2 antibody was obtained from Dr E. Mechetner (Ingenex Inc. Menlo Park, CA, U.S.A.). All other chemicals were supplied by BDH or Sigma (Poole, Dorset).

Statistics

Results are expressed as mean $\pm$ s.e.mean (n). For statistics relating to J_{net}, individual values of net flux from paired monolayers were used. For unidirectional fluxes and permeabilities individual values calculated from data derived from paired monolayers (above) were used. Statistical analysis was performed by Student's unpaired t test or one-way analysis of variance (ANOVA) with a Bonferroni post test for multiple comparisons (Graph-Pad Instat, SanDiego, U.S.A.). Kinetic constants for Michaelis-Menten kinetics were calculated by non-linear regression with the method of least squares (FigP, Biosoft, Cambridge, U.K.).

Results

Transepithelial ciprofloxacin fluxes and cellular uptake in Caco-2 epithelia

Figure 1 demonstrates that in control conditions ciprofloxacin is secreted from basal to apical solutions in Caco-2 epithelia; that is basal to apical flux, $J_{b\text{-}a}$, exceeded apical to basal flux, $J_{a\text{-}b}$, of [^{14}C]-ciprofloxacin, consistent with previous data (Griffiths *et al*., 1993; 1994). This secretory net flux (J_{net}) was not linearly dependent on ciprofloxacin concentration. J_{net} demonstrated saturation kinetics (not shown) with half-maximal saturation being observed at 2.87 $\pm$ 0.11 mM and a maximal secretory capacity of 75.4 $\pm$ 1.6 nmol cm^{-2} h^{-1} (data from 30 monolayers). With an external ciprofloxacin concentration of 0.1 mM there was also a marked asymmetry in steady-state cellular ciprofloxacin uptake from the basolateral surface (0.70 $\pm$ 0.07 mM, n = 47) compared with that at the apical surface (0.10 $\pm$ 0.02 mM, n = 46). The apparent cell to medium ratio (C/M) was 7.0 for loading from the basal solution, demonstrating that there was marked accumulation at the basolateral membrane, and this value is comparable to those previously found (C/M 6.4; Griffiths *et al*., 1994).

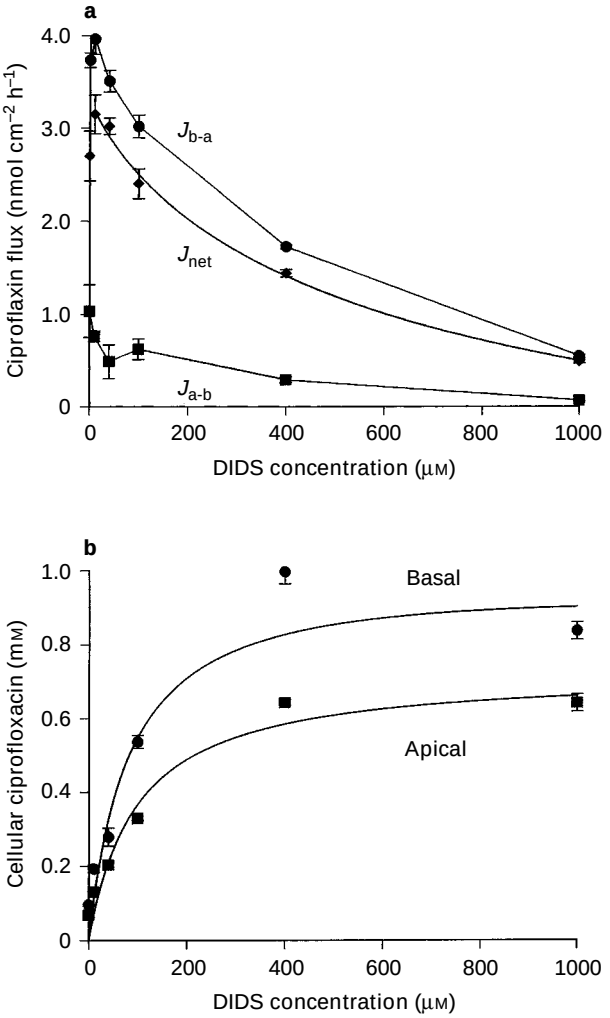

Figure 1 Concentration-dependence of inhibition of ciprofloxacin transepithelial fluxes and cellular uptake by DIDS. (a) Transepithelial fluxes of ciprofloxacin (at 0.1 mM) alone and in the presence of DIDS were determined for apical to basal flux ($J_{a\text{-}b}$) and basal to apical flux ($J_{b\text{-}a}$). Net secretory flux J_{net} shown as ($J_{b\text{-}a} - J_{a\text{-}b}$). Least-square fit to J_{net} (linear dose vs effect) is shown, $K_{0.5}$ for inhibition of ciprofloxacin by DIDS = 385.2 $\pm$ 68.3 μM. Increasing doses of DIDS were present in both apical and basolateral compartments. n = 3 epithelia or pairs per data point. (b) Concentration-dependence of DIDS on steady-state values of cellular ciprofloxacin measured across apical or basal cell borders. Michaelis-Menten least-square fits give $K_{0.5}$ values for half-maximal stimulation of cellular uptakes by DIDS (99.08 $\pm$ 31.18 μM across apical membrane and 85.90 $\pm$ 55.37 μM across the basolateral membrane). Details as in (a).

Effect of substrates/inhibitors of hepatic and renal organic anion transporters on the transepithelial transport and cellular uptake of ciprofloxacin

Substrates of the three renal basolateral organic anion transport systems (Ullrich & Rumrich, 1988) were tested for their ability to act as competitive inhibitors of intestinal ciprofloxacin secretion (Table 1). Sulphate, oxalate and thiosulphate, all at 10 mM, (K_i values for the renal sulphate transport system, 1.4, 1.1 and 0.3 mM, respectively) did not decrease ciprofloxacin secretion. Similarly succinate ($K_i = 0.1$ mM) and PAH ($K_i = 1.3$ mM) as substrates for the renal dicarboxylate transporter were also without effect on ciprofloxacin secretion. Probenecid and PAH are also substrates for the PAH transport system (K_i values 0.05 and 0.08 mM, see Ullrich & Rumrich, 1988). Probenecid was also without effect on ciprofloxacin net secretion across Caco-2 epithelial layers (Table 1). Uptake of ciprofloxacin across either the apical or basolateral surfaces was not changed (data not shown).

However, the organic anion exchange inhibitor 4,4′-diisothiocyanostilbene-2-2′-disulphonic acid (DIDS) (Ullrich & Rumrich, 1988) gave a concentration-dependent inhibition of ciprofloxacin transport and stimulation of ciprofloxacin uptake (Figure 1). Net secretion of ciprofloxacin was significantly inhibited at a concentration of 0.4 mM DIDS, through a decrease in both $J_{b\text{-}a}$ (control 3.74 ± 0.08 nmol cm^{-2} h^{-1}; plus DIDS 1.72 ± 0.02 nmol cm^{-2} h^{-1}, both $n = 3$, $P < 0.05$) and $J_{a\text{-}b}$ (1.01 ± 0.29 nmol cm^{-2} h^{-1}; plus DIDS 0.29 ± 0.03 nmol cm^{-2}, h^{-1}, both $n = 3$, $P < 0.05$). Mannitol flux measured concurrently was unchanged ($J_{b\text{-}a}$ plus 0.4 mM DIDS was $99.0 \pm 0.1\%$ of control values, $n = 3$). The IC_{50} for DIDS inhibition of ciprofloxacin secretion was 385.2 ± 68.3 μM. This is of low affinity compared to DIDS inhibition of renal organic anion secretion (Ullrich & Rumrich, 1988). Steady-state cellular uptake of ciprofloxacin increased significantly in the presence of 40 μM DIDS, with loading across both apical (control 0.07 ± 0.01 μM; plus DIDS 0.21 ± 0.01 μM, both $n = 3$, $P < 0.05$) and basolateral (control $0.10 \pm 0.00(3)$; plus DIDS 0.28 ± 0.02 μM, both $n = 3$, $P < 0.05$) membrane surfaces. Half-maximal stimulation of ciprofloxacin uptake at the apical and basolateral membrane surfaces occurred at a lower concentration than that for half-maximal inhibition of J_{net} (99.08 ± 31.18 μM and 85.90 ± 55.37 μM across the apical and basolateral membranes, respectively).

As shown in Figure 2 and Table 2, on addition of increasing concentrations of DIDS, the permeability of ciprofloxacin decreased across both apical and basolateral membranes in both directions. However, exit permeabilities from the cell were inhibited to a greater extent than entry permeabilities into the cell. The concentration of DIDS at which half-maximal inhibition occurred was 13.91 ± 2.66 μM in the case of $P_{c\text{-}a}$ and 12.91 ± 1.21 μM in the case of $P_{c\text{-}b}$, whilst half-maximal inhibition of $P_{b\text{-}c}$ and $P_{a\text{-}c}$ occurred at a much higher concentration of DIDS (see Figure 2). This indicates that DIDS had a more potent and proportionately greater inhibitory effect at the membrane surfaces exposed to the cytosol than at those exposed to the extracellular medium.

Table 1 Effect of competitive substrates/inhibitors for the renal organic anion and cation transporters on intestinal ciprofloxacin secretion

Inhibitor (n, N)	J_{net} *ciprofloxacin secretion*
Sulphate (5,2)	1.01 ± 0.06
Succinate (6,2)	1.04 ± 0.035
PAH (8,3)	1.01 ± 0.09
Oxalate (3,1)	0.98 ± 0.02
Thiosulphate (5,2)	1.06 ± 0.05
Probenecid (6,2)	1.20 ± 0.14
NMN (5,2)	1.12 ± 0.14
TEA (4,1)	1.19 ± 0.10
Probenecid + NMN (3,1)	0.78 ± 0.11
Verapamil (100 μM) (10,4)	0.32 ± 0.06**

Net secretory flux J_{net} of ciprofloxacin was determined at 0.1 mM ciprofloxacin (control = 1.0). Substrates/inhibitors were present at 10mM in both the basolateral and apical bathing solutions except where indicated. Data are the mean ± s.e.mean of n separate epithelial pairs in N experiments (shown in parentheses). At 0.1 mM net ciprofloxacin secretion from basal to apical surfaces (J_{net}) was 2.7 ± 0.1 nmol cm^{-2} h^{-1} $n = 34$, $N = 11$. Significantly different from control values, **$P < 0.01$.

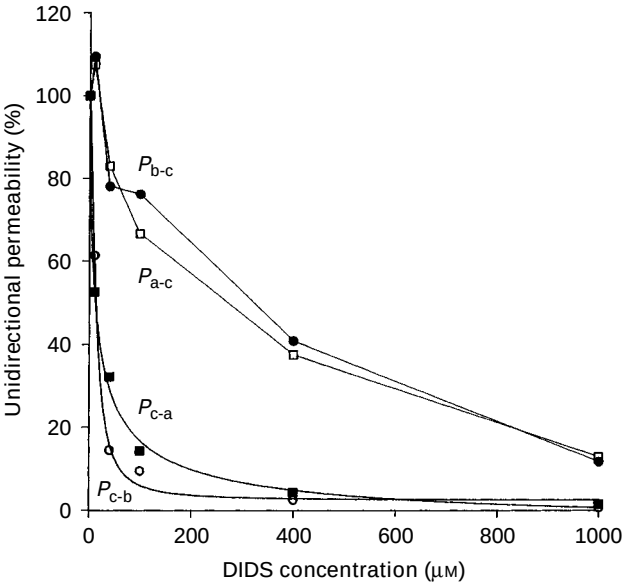

Figure 2 Effect of DIDS on the unidirectional permeabilities of ciprofloxacin at the apical and basolateral cell borders. Unidirectional permeabilities were calculated by use of the ratio of apical/basal ciprofloxacin uptake and total cellular ciprofloxacin from Figure 1 as described in Methods. Permeabilities are expressed as % change from control values. Fitted curves to $P_{c\text{-}a}$ and $P_{c\text{-}b}$ (linear dose vs effect) provide $K_{0.5}$ values for inhibition of ciprofloxacin by DIDS (13.91 ± 2.66 μM and 12.91 ± 1.21 μM, respectively). $n = 3$ epithelial pairs per data point.

Table 2 Effect of DIDS on the calculated unidirectional permeabilities (P) at the apical and basolateral cell borders of Caco-2 cell monolayers

	$P_{a\text{-}c}$	$P_{c\text{-}a}$	$P_{c\text{-}b}$	$P_{b\text{-}c}$	$P_{a\text{-}c}/P_{c\text{-}a}$	$P_{b\text{-}c}/P_{c\text{-}b}$
Control	3.72 ± 0.11	3.90 ± 0.20	1.58 ± 0.51	5.30 ± 0.58	0.95	3.35
DIDS	1.40 ± 0.07*	$0.17 \pm 0.00(4)$*	$0.04 \pm 0.00(4)$*	2.17 ± 0.03*	8.24	54.29

Ciprofloxacin fluxes were determined alone (0.1 mM unlabelled ciprofloxacin) and in the presence of 400 μM DIDS in both apical and basolateral compartments. Unidirectional permeabilities were calculated as described in Methods and are expressed in cm h^{-1} (x 10^{-2}). Values of total intracellular ciprofloxacin concentration were taken from Figure 3b and it was assumed that cyprofloxacin was not sequestered and was evenly distributed in the cytosol (see Discussion). $n = 7-8$. *$P < 0.05$, significantly different from control.

Figure 3 shows the effectiveness of 0.4 mM DIDS when added to either apical or basolateral bathing solutions alone, or at both epithelial surfaces simultaneously on inhibition of ciprofloxacin transepithelial fluxes and uptake. When DIDS was present in the apical compartment there was no significant decrease in either $J_{b\text{-}a}$ or $J_{a\text{-}b}$. However, when DIDS was present in the basolateral compartment there was a 32% decrease in ciprofloxacin J_{net} (control 2.06 ± 0.15 nmol cm^{-2} h^{-1}; DIDS basally 1.40 ± 0.09 nmol cm^{-2} h^{-1}, both $n=8$, $P<0.05$). When ciprofloxacin was present in both apical and basolateral compartments there was a 50% decrease in ciprofloxacin J_{net} as compared to control values (DIDS apically and basally 1.02 ± 0.07 nmol cm^{-2} h^{-1}, $n=8$, vs control $P<0.05$). This decrease in net secretion was due to a large decrease in $J_{b\text{-}a}$ and a smaller decrease in $J_{a\text{-}b}$. There was a marked increase in cellular ciprofloxacin with basolateral loading and a smaller increase from the apical solution when DIDS was present in the basolateral or both compartments but not when present in the apical compartment only (see Figure 3b). The related compound SITS (4-acetamido-4′-isothiocyanostilbene 2,2′-disuphonic acid) was without significant inhibitory effect at 0.4 mM (J_{net} was 109 ± 9% of control values, $n=3$).

DIDS acts by irreversibly blocking lysine residues (Cabantchik & Rothstein, 1974), 4′4-dinitro-2,2′-disulphonic acid (DNDS) acts in the same manner but its effect is reversible. DNDS (0.4 mM) had no effect on the net secretion of ciprofloxacin (control 2.04 ± 0.15 nmol cm^{-2} h^{-1}, $n=4$; plus DNDS 1.45 ± 0.53 nmol cm^{-2} h^{-1}, $n=3$, $P<0.05$). Uptake across the apical (control 0.10 ± 0.01 mM, $n=4$; plus DNDS 1.45 ± 0.53 mM, $n=5$, $P<0.05$) and basolateral (control 0.63 ± 0.13 mM, $n=4$; plus DNDS 1.45 ± 0.53 mM, $n=3$, $P<0.05$) membrane faces was also unaffected.

Effect of substrates/inhibitors for organic cation transporters and P-glycoprotein on the transepithelial transport and cellular uptake of ciprofloxacin

Two archetypal cation substrates, N-′methylnicotinamide (NMN) and tetraethylammonium (TEA) (Ullrich *et al*., 1993a,b) were tested on transport and cellular uptake of ciprofloxacin in Caco-2 cell monolayers (Table 1). Neither 10 mM NMN nor 10 mM TEA (added to both compartments) had any significant inhibitory effects on transport or cellular uptake of ciprofloxacin (Table 1). There was also no effect of the inclusion of both an inhibitor of renal organic anion transport, probenecid, and a substrate for renal organic cation transport, NMN on ciprofloxacin transport (Table 1).

However, 100 μM verapamil, which in addition to being a substrate for the renal organic cation transporter (Ullrich *et al*., 1993b), is also a competitive inhibitor for P-glycoprotein (Hunter *et al*., 1993c), caused a significant decrease in net ciprofloxacin secretion (Table 1). To investigate this inhibitory effect more fully, the dose-dependency of verapamil inhibition

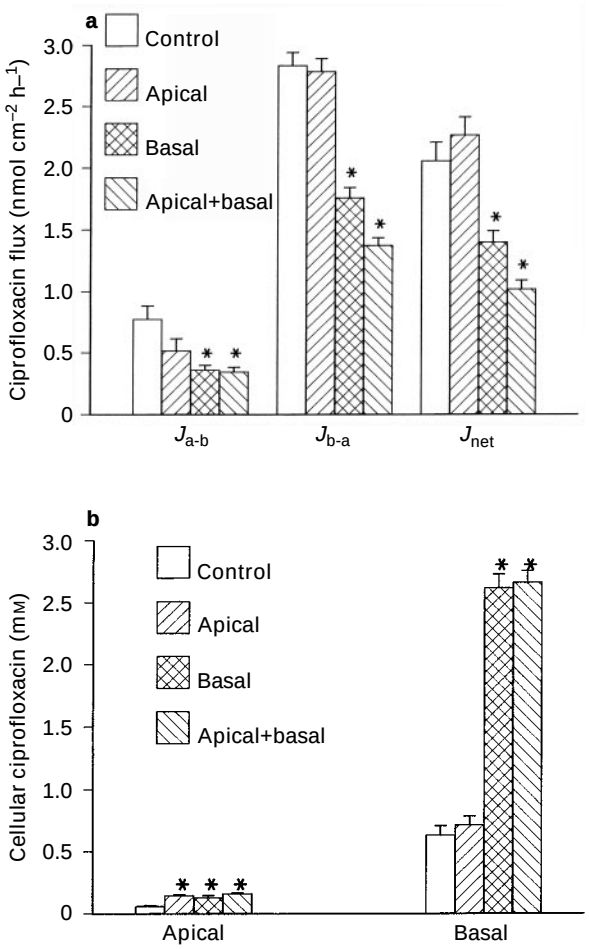

Figure 3 Transepithelial ciprofloxacin fluxes and uptake in the presence of DIDS (0.4 mM) at either the apical or basolateral membrane surface. (a) Transepithelial ciprofloxacin (0.1 mM) fluxes measured alone and in the presence of 0.4 mM DIDS in either the apical or basolateral compartment or both compartments simultaneously. J_{net} shown at ($J_{b\text{-}a} - J_{a\text{-}b}$). $n=8$ epithelia or pairs per data point. (b) Cellular ciprofloxacin from apical or basal bathing solutions in the presence and absence of DIDS (0.4 mM). *Significantly different from control data, $P<0.05$.

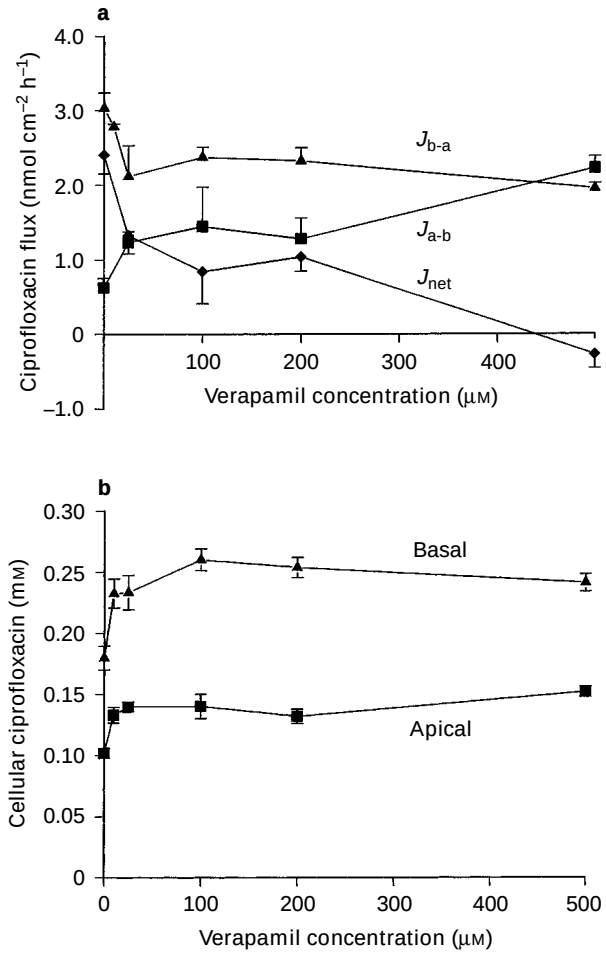

Figure 4 Concentration-dependence of inhibition of ciprofloxacin transepithelial fluxes and uptake by verapamil. (a) Transepithelial fluxes of ciprofloxacin (0.1 mM) alone and in the presence of verapamil were determined for apical basal flux ($J_{a\text{-}b}$) and basal to apical flux ($J_{b\text{-}a}$). Net secretory flux ($J_{net} = J_{b\text{-}a} - J_{a\text{-}b}$). Increasing doses of verapamil were present in both apical and basolateral compartments. $n=5$–6 epithelia or 5 pairs per data point. (b) Concentration-dependence on verapamil on cellular ciprofloxacin measured across apical or basolateral surfaces. Other details as in (a).

on ciprofloxacin transepithelial transport and accumulation was investigated (see Figure 4). Figure 4a shows the dose-dependence of verapamil on [^{14}C]-ciprofloxacin. At 100 μM verapamil there was a 64.9% decrease in net ciprofloxacin secretion, with a concomitant increase in $J_{a\text{-}b}$ (control 0.63 ± 0.13 nmol cm^{-2} h^{-1}, $n=6$; plus verapamil 1.45 ± 0.53 nmol cm^{-2} h^{-1}, $n=5$, $P<0.05$) and decrease in $J_{b\text{-}a}$ (control 3.03 ± 0.21 nmol cm^{-2} h^{-1}, $n=6$; plus verapamil 2.29 ± 0.14 nmol cm^{-2} h^{-1}, $n=5$, $P<0.05$).

This contrasts with the effect of verapamil on the P-glycoprotein substrate vinblastine, a P-glycoprotein substrate which is subject to a net secretion in Caco-2 cell monolayers (Hunter *et al.*, 1993a,c). At 100 μM verapamil there was complete abolition of vinblastine net flux due to a decrease in $J_{b\text{-}a}$ and an increase in $J_{a\text{-}b}$ (control 0.61 ± 0.06 pmol cm^{-2} h^{-1}, $n=4$; plus verapamil 0.04 ± 0.06 nmol cm^{-2} h^{-1}, $n=3$, $P>0.05$). However, verapamil failed to cause complete inhibition of ciprofloxacin net secretion except at high and probably cytotoxic levels (500 μM), where there was a large increase in $J_{a\text{-}b}$. This was mirrored by a large increase in simultaneously measured mannitol $J_{a\text{-}b}$ (control 0.73 ± 0.13 nmol cm^{-2} h^{-1}; plus 500 μM verapamil, 1.57 ± 0.22 nmol cm^{-2} h^{-1}, both $n=6$, $P<0.05$, at 0.1 mM mannitol).

Verapamil (100 μM) also caused a maximal increase of 44% in basolateral uptake of ciprofloxacin (control 0.18 ± 0.01 mM; plus verapamil 0.26 ± 0.01 mM, both $n=6$, $P<0.05$) (see Figure 4b). In comparison, 100 μM verapamil caused a 309% increase in basolateral uptake of vinblastine (control 22.3 ± 2.46 μM, $n=4$; plus verapamil 69.00 ± 1.10 μM, $n=3$, $P<0.05$).

Unidirectional permeabilities (Figure 5 and Table 3) were calculated across each cell border by use of the extracellular or apparent cellular concentrations as appropriate. As shown in Figure 5 and Table 3, ciprofloxacin $P_{c\text{-}a}$ was inhibited 50% by 100 μM verapamil, whilst $P_{c\text{-}b}$ was increased. $P_{a\text{-}c}$ and $P_{b\text{-}c}$ were largely unaffected by verapamil. These data contrast with the inhibitory effect of DIDS on ciprofloxacin transport (Figure 2 and Table 2). DIDS decreased all four permeabilities, the greatest effect being on $P_{c\text{-}a}$ and $P_{c\text{-}b}$. In comparison, verapamil only affected $P_{c\text{-}b}$ and $P_{c\text{-}a}$, $P_{c\text{-}b}$ being stimulated rather then inhibited.

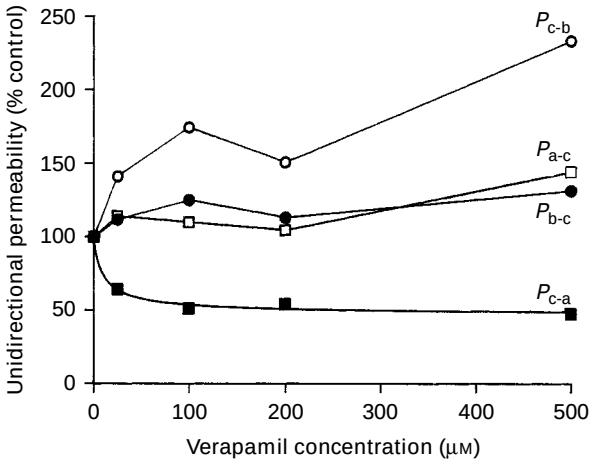

Figure 5 Effect of verapamil on the calculated permeabilities (P) of ciprofloxacin at the apical and basolateral cell borders. Unidirectional permeabilities were calculated by use of the ratio of apical/basal ciprofloxacin uptake and total cellular ciprofloxacin from Figure 4 as described in Methods. Permeabilities are expressed as % change from control values. $n=5$ epithelial pairs per data point.

Transport of the P-glycoprotein substrate vinblastine was inhibited by verapamil with a similar pattern to ciprofloxacin i.e. decrease in $P_{c\text{-}a}$, increase $P_{c\text{-}b}$ (Table 3). However, verapamil caused a reduction in $P_{c\text{-}a}$ to 18% of its control value, consistent with inhibition of P-glycoprotein at this membrane and a much larger stimulation of $P_{c\text{-}b}$ at 100 μM as compared to its effect on ciprofloxacin transport.

[^{3}H]-vinblastine flux (10 mM vinblastine present in both apical and basal solutions) was measured in the presence and absence (control) of 3 mM ciprofloxacin. There was no significant difference in net secretion of vinblastine in the presence of 3 mM ciprofloxacin (J_{net} control $= 1.08$ nmol cm^{-2} h^{-1}, $n=7$; plus 3 mM ciprofloxacin $J_{net} = 0.93 \pm 0.06$ nmol cm^{-2} h^{-1}, NS). There was a significant reduction in the cellular uptake of vinblastine across both the apical and basolateral membrane surfaces (apical accumulation from 89 ± 6.5 to 62.4 ± 2.7 μM $n=7$, $P<0.05$; basal accumulation from 178.0 ± 17.2 to 97.3 ± 3.1 μM, $n=7$, $P<0.05$). This inhibition of uptake contrasts with P-glycoprotein inhibition by verapamil, which caused an increase in the cellular uptake of vinblastine (see above).

In MDCK I and II epithelial layers ciprofloxacin (1 μM) was not subject to secretion, despite a substantial net secretion of vinblastine being observed; rather there was a small net absorption in these two cells (see Figure 6a and b). An explanation could be that ciprofloxacin does not access the cytosol of MDCK cells across the basolateral face. However, Table 4 shows that ciprofloxacin was accumulated by MDCK cells at the basolateral cell surface in a similar fashion to that seen in Caco-2 epithelial cells.

Though DIDS has a marked inhibitory action on ciprofloxacin secretion, it has no effect on vinblastine secretion (McEwan *et al.*, 1992). DIDS (0.4 mM) had no inhibitory action on the transport of vinblastine (10 nM) when present in both the apical and basal bathing solutions (control vinblastine secretion $J_{net} = 0.585 \pm 0.007$ nmol mc^{-2} h^{-1}; plus DIDS, $J_{net} = 0.681 \pm 0.005$ nmol cm^{-2} h^{-1}, $n=4$) and only a small effect on vinblastine uptake (not shown). These data provide clear pharmacological evidence for a separate mechanistic basis for ciprofloxacin secretion by Caco-2 epithelia.

The effects of the anti-human P-glycoprotein antibodies UIC2 or MRK16 are shown in Figure 7. Secretory ciprofloxacin $P_{b\text{-}a}$ was unchanged from control values. In contrast, with both anti-P-glycoprotein antibodies there was a significant decrease in secretory vinblastine permeability $P_{b\text{-}a}$ measured concurrently (Figure 7a,b).

Table 3 Effect of verapamil on the calculated unidirectional permeabilities (P) at the apical and basolateral cell borders of Caco-2 cell monolayers

	$P_{a\text{-}c}$	$P_{c\text{-}a}$	$P_{c\text{-}b}$	$P_{b\text{-}c}$	$P_{a\text{-}c}/P_{c\text{-}a}$	$P_{b\text{-}c}/P_{c\text{-}b}$
Ciprofloxacin						
Control	2.40 ± 0.26	1.70 ± 0.16	0.63 ± 0.15	4.17 ± 0.32	1.41	6.62
Verapamil	2.64 ± 0.54	0.87 ± 0.04*	1.10 ± 0.41*	5.22 ± 1.26	3.03	4.77
Vinblastine						
Control	6.05 ± 0.53	2.82 ± 0.27	0.11 ± 0.01	10.30 ± 0.96	2.14	93.6
Verapamil	9.99 ± 0.76*	0.53 ± 0.03*	0.46 ± 0.06*	12.56 ± 1.27	18.84	27.3

Cipofloxacin (90.1 mM) and vinblastine (6.25 nM) bidirectional fluxes were determined alone and in the presence of 100 μM verapamil in the apical and basolateral compartments. Unidirectional permeabilities were calculated as described in Methods and are expressed in cm h^{-1} (x 10^{-2}). Values of total intracellular ciprofloxacin concentration were taken from Figure 4b and a uniform distribution in the cytosol was assumed (see Discussion). $n=7-8$. *$P<0.05$, significantly different from control.

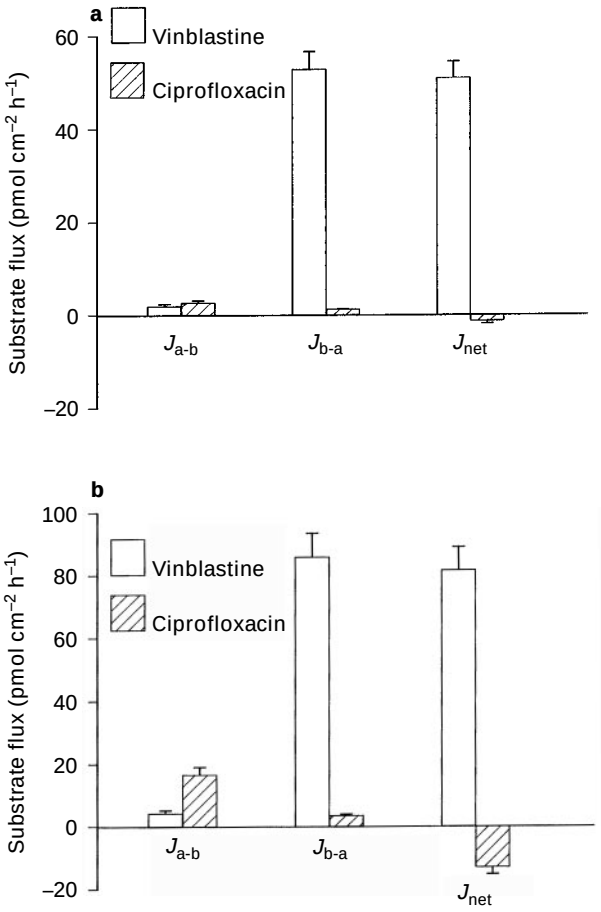

Figure 6 Transepithelial fluxes of vinblastine and ciprofloxacin in MDCK strains I and II. (a) Transepithelial and net secretory flux of vinblastine and ciprofloxacin (both 10 μM) measured in separate MDCK strain I monolayer. $J_{net} = J_{b\text{-}a} - J_{a\text{-}b}$. $n = 4-5$ epithelia or 4 epithelial pairs per data point. (b) Transepithelial and net secretory flux vinblastine and ciprofloxacin (both at 10 μM) measured in separate MDCK strain II monolayer. $n = 4-5$ epithelia or 4 epithelial pairs per data point.

Table 4 Steady-state cellular uptakes of vinblastine and ciprofloxacin in the cell lines MDCK I and MDCK II

	Apical uptake (μM)	*Basal uptake* (μM)	*Ratio A/B*
MDCK I			
Vinblastine	19.28 ± 0.86	188.67 ± 19.00	0.10
Ciprofloxacin	0.85 ± 0.08	3.59 ± 0.07	0.24
MDCK II			
Vinblastine	30.28 ± 1.83	63.01 ± 7.37	0.48
Ciprofloxacin	1.88 ± 0.19	2.62 ± 0.14	0.72

Uptakes were determined at 10 μM vinblastine or μM ciprofloxacin and are expressed in μM. $n = 4-5$.

Relationship between secretory ciprofloxacin transporter and BSP transport and glutathione-conjugate transport

Substrates for the Na^+-independent liver bile acid transporter (taurocholate and bromosulphophthalein (BSP)), were tested for their ability to inhibit ciprofloxacin secretion. Ciprofloxacin secretion (at 0.1 mM) was maintained in the presence of taurocholate (89 ± 11% ($n = 6$) and 110 ± 16% ($n = 3$) vs controls at 50 and 100 μM, respectively) or BSP (126 ± 20% ($n = 6$) and 130 ± 11% ($n = 3$) vs control values at 50 and 100 μM, BSP, respectively). Similarly, ciprofloxacin secretion was maintained (87 ± 7%, $n = 6$ versus controls) despite the inclusion of saturating concentrations of 1-chloro-2,4-dinitrobenzene (CDNB) which is conjugated to glutathione and excreted as a conjugate (Elferink *et al*., 1993).

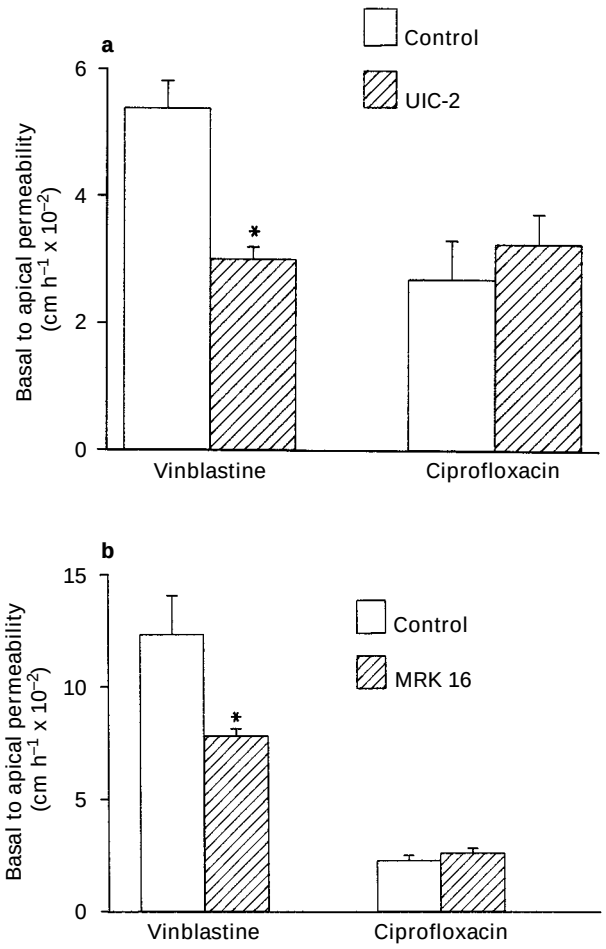

Figure 7 Effect of the anti-P-glycoprotein monoclonal antibodies (a) UIC2 and (b) MRK16 on the basolateral to apical permeability of vinblastine and ciprofloxacin. Transepithelial permeabilities of vinblastine (30 nM) and ciprofloxacin (10 μM), (a) in the presence of 25 μg ml^{-1} control antibody IgG1 and 25 μg ml^{-1} MRK16. Cells were preincubated with either antibody on the apical surface for 2 h before the flux was determined. $n = 4$ epithelia per data point. *Significantly different from control values, $P < 0.05$.

Effect of intracellular and extracellular pH changes on the transepithelial transport and uptake of ciprofloxacin

Altering the medium pH to either 6.0/5.5 or 8.5 at the apical membrane had only a small effect upon net secretion of ciprofloxacin (measured at 0.1 mM total medium ciprofloxacin); it increased to 145 ± 22% and 145 ± 23% of control values ($n = 9$), through an increase in $J_{b\text{-}a}$ (not shown). Altering the pH of the basolateral solution to 5.5/6.0 or 8.5 was without effect on ciprofloxacin secretion (94 ± 12% and 119 ± 11% of control values, $n = 9$). Cellular accumulation of ciprofloxacin was similarly unaffected (data not shown). This lack of effect of medium pH contrasts with that seen with H^+/peptide symport (Thwaites *et al*., 1993a,b).

When cytosolic pH was made acidic (Na^+-free media, NH_4Cl prepulse; see Methods) no significant effect was seen upon the net secretion of ciprofloxacin by Caco-2 epithelial layers, measured at medium concentration of 5 μM ciprofloxacin ($J_{net} = 1.76$ nmol cm^{-2} h^{-1} (control); after acidification, $J_{net} = 1.54$ nmol cm^{-2} h^{-1}, NS, $n = 3$ epithelial pairs). Cellular acidification slightly increased ciprofloxacin accumulation within the cell from the apical medium (5.7 ± 0.1 μM to 7.3 ± 0.4 μM, $P < 0.05$, $n = 3$ pairs), whilst decreasing accumu-

lation from the basal medium (22.1 ± 0.8 μM to 16.0 ± 1.5 μM, $P < 0.05$, $n = 3$ pairs).

Dependence of the transepithelial transport and cellular uptake of ciprofloxacin on medium Na^+, K^+ and Cl^-

In order to test whether transport of ciprofloxacin is coupled (directly or indirectly) to the cation and anion gradients that exist across the apical and basolateral cell membranes the effect of medium ion (sodium, potassium and chloride) replacement was investigated. Ciprofloxacin secretion (at 0.1 mM) was maintained in the absence of Na^+ ($106 \pm 11\%$ ($n = 3$) of control values ($n = 4$)), K^+ ($96 \pm 28\%$ of controls) or Cl^- ($72 \pm 8\%$ of controls).

Furthermore, preincubation of the Caco-2 cells with 0.1 mM ouabain in the basal solution for 1 h before flux determination, in order to inhibit Na-K pump function and to dissipate cellular ion gradients, resulted in no change in ciprofloxacin $J_{b\text{-}a}$ (control 2.92 ± 0.15 nmol cm^{-2} h^{-1}, $n = 8$; plus ouabain 2.94 ± 0.24 nmol cm^{-2} h^{-1}, $n = 7$, $P > 0.05$). Apical to basolateral flux and J_{net} were also not altered (data not shown). Cellular uptake of ciprofloxacin was not altered on preincubation with ouabain at the apical membrane (data not shown) but at the basolateral membrane increased by 27% compared to controls ($n = 7$, $P < 0.05$).

Discussion

The existence of an ATP-dependent saturable net intestinal secretion of ciprofloxacin and other fluorquinolone antibiotics (Griffiths *et al*., 1993; 1994) will affect both the oral bioavailability, distribution and pharmacokinetics of these agents. The present study was designed to investigate the mechanism of ciprofloxacin secretion in the human intestinal Caco-2 cell system.

Iseki *et al*. (1992) have investigated the oral bioavailability of fluoroquinolones such as enoxacin by studying uptake in rat isolated intestinal brush-border membrane vesicles. Enoxacin uptake was higher at pH 5.5 than pH 7.5, suggesting that uptake of the cationic form predominates in potential-dependent uptake. This diffusive potential-dependent uptake was inhibited by ciprofloxacin (Hirano *et al*., 1995a). Intestinal absorptive transport ($J_{a\text{-}b}$) for the fluoroquinolones enoxacin and ofloxacin has been characterized. Absorption of ofloxacin was highest at pH 7 (maximal pH of zwitterionic species) and decreased at pH 5 and pH 8 (Rabbaa *et al*., 1995). Prieto *et al*. (1988), fitted a saturable Michaelis-Menten process to the intestinal absorption of ofloxacin rather that a simple diffusion mechanism. These data suggest mediation of the cationic form of fluoroquinolones at the apical brush-border membrane. However, as with several fluoroquinolones, ofloxacin is subject to secretion in the intestine (Rabbaa *et al*., 1995), thus it is possible that absorption is limited by secretion in the opposite direction, rather than there being a specific saturable absorptive process.

Because ciprofloxacin is zwitterionic, at pH 7.4 it will exist predominantly as a neutral molecule. At this isoelective point its solubility will be lowest and lipophilicity highest. At pH values below 6.5, ciprofloxacin will exist predominantly in the cationic form and at pH values greater than 8.5 the anionic form will predominate. In the present studies, there was no effect of changing the pH of the basolateral bathing solution (between pH 5.5 and pH 8.5) on the net secretion of ciprofloxacin, surprisingly demonstrating that the ionization state of ciprofloxacin does not affect its secretory transport. *In vivo* studies in rats have also shown that alteration of the basolateral surface perfusate with pH 6.0, 7.4 and 8.5 caused no change in transepithelial elimination of ciprofloxacin (Rubinstein *et al*., 1994). The absorption kinetics (i.e. $J_{a\text{-}b}$) of ciprofloxacin do not appear to show any sign of saturation and $J_{a\text{-}b}$ was significantly reduced when the apical medium was made acidic or alkaline, giving a small stimulation of net secretion of ciprofloxacin. This would be expected if the absorptive transport was determined by simple diffusion, as any change in pH from 7.4 in either acidic or alkali directions will reduce the lipophilicity of the molecule.

Net secretion of ciprofloxacin was not dependent on sodium, chloride or potassium, demonstrating a lack of direct coupling with these ions. There was no effect of a preincubation with ouabain on secretion, confirming that normal cation gradients are not important for ciprofloxacin secretion. This also demonstrates that ciprofloxacin-secretion is not dependent on membrane potential (Hirano *et al*., 1994a,b).

In the kidney, ciprofloxacin is secreted via both the organic anion and cation transporter systems (Sorgel & Kinzig, 1993b; Ullrich *et al*., 1993a; Hirano *et al*., 1994b). Fluoroquinolones have previously been shown to be transported via organic anion transporters at sites other than the kidney. J774 macrophages secrete norfloxacin after intracellular loading, this transport being inhibited by probenecid, suggesting that it is transported via organic anion transporters (Cao *et al*., 1992). Fleroxacin is secreted in a cerebrospinal fluid (CSF) to blood direction by a probenecid inhibitable organic anion transporter in the rat choroid plexus (Ooie *et al*., 1996).

In human intestinal Caco-2 cells, secretion of ciprofloxacin appears to be distinct from renal organic anion transport systems. This is demonstrated by lack of inhibition of a range of substrates and inhibitors of the renal organic anion transport systems. *In vivo* studies in man have also shown that probenecid reduces renal clearance of ciprofloxacin by inhibiting tubular secretion, whilst having no effect on non-renal clearance (Jaehde *et al*., 1989). Rubinstein *et al*. (1994) showed that there was no effect of probenecid on the intestinal transepithelial elimination of ciprofloxacin in rat. Thus there is good agreement between these studies and the Caco-2 model.

The liver is now known to express a range of organic anion transport systems, one of which, the Na^+-independent basolateral organic anion transporting polypeptide (oatp) may be expressed in proximal colon (Jacquemin *et al*., 1994). Given the colonic origin of Caco-2 cells, it is possible that these cells also express this transporter. However, two archetypal substrates for this transporter had no effect on ciprofloxacin transport or uptake. Recently, the multidrug resistance associated protein (MRP) has been identified which is responsible for secretion of glutathione conjugates (Jedlitschky *et al*., 1994) and also a number of hydrophilic drugs (Grant *et al*., 1994). MRP is found in many tissues (Flens *et al*., 1996) and is expressed in Caco-2 cells at low levels (Evers *et al*., 1996). The ATP-dependent transport of doxorubicin can be competitively inhibited by glutathione conjugates and vinblastine, doxorubicin and daunomycin stimulate ATP hydrolysis by DNP-SG ATPase (Awasthi *et al*., 1994). Ciprofloxacin secretion is unaffected by the inclusion of saturation concentrations of CDNB, which is subject to glutathione-S-conjugation and secretion (Elferink *et al*., 1993). In addition, probenecid inhibits glutathione-S-conjugate transport (Evers *et al*., 1996) but has no effect on the transport of ciprofloxacin.

In the kidney, fluoroquinolone secretion is not only mediated via the organic anion system but there is also an interaction of fluoroquinolones with the organic cation system (Sorgel & Kinzig, 1993b; Ullrich *et al*., 1993a). Ofloxacin interacts with the organic cation/H^+ antiporter system in rat renal brush border membranes (Okano *et al*., 1990), whilst enoxacin interacts with the organic cation transport system in the brush-border membrane of rat renal cortex (Hirano, 1995b). Secretion of the β-adrenoceptor blocking drug celiprolol in Caco-2 cells shares characteristics with renal organic cation transport, suggesting that a putative organic cation/H^+ exchanger may be involved (Karlsson *et al*., 1993). The lack of effect of variation in both medium and intracellular pH combined with the absence of inhibition of ciprofloxacin net secretion in the presence of either N′methylnicotinamide or tetraethylammonium (2 archetypal organic cation substrates, see Pritchard & Miller, 1993) in Caco-2 cells, strongly suggest

that intestinal ciprofloxacin secretion is not mediated by transporters similar to the renal organic cation transporters.

Another route for the secretion of hydrophobic drugs is the ATP-driven efflux pump P-glycoprotein. The presence of P-glycoprotein in Caco-2 cells is well-documented. Monoclonal antibodies have been used to identify an appropriate protein by Western-blotting (Peters & Roelofs, 1992), whilst in immunocytochemical studies (with MRK16) the brush-border of Caco-2 cells have been stained (Hunter *et al*., 1993c). There is extensive evidence presented here to suggest that the secretion of ciprofloxacin is not mediated by P-glycoprotein. The J_{max} for the net secretion of ciprofloxacin (75.4 ± 1.6 nmol cm^{-2} h^{-1}) was greatly in excess of that exhibited by known P-glycoprotein substrates in Caco-2 cells (vinblastine J_{max}, 1.3 ± 0.3 nmol cm^{-2} h^{-1}; Hunter *et al*., 1993c), suggesting it is transported by a distinct transport process. Substrates for a single common transport system should have identical J_{max} values.

Further evidence suggests that ciprofloxacin transport is distinct from P-glycoprotein. The renal cell lines MDCKI and MDCKII preferentially transport vinblastine in a secretory direction (Hunter *et al*., 1993b). Wild-type MDCK cells express P-glycoprotein demonstrated by immunoprecipitation with rabbit polyclonal antiserum prepared against C-terminal fragment of P-glycoprotein and their ability to secrete vinblastine, vincristine, daunomycin and actinomycin D (Horio *et al*., 1989). Ciprofloxacin is not secreted by MDCK cells, instead there is a small net absorption. However, the cells accumulate ciprofloxacin across the basolateral membrane, suggesting a mediated transport mechanism at the membrane face and excluding the possibility of a non cytosolic access as a reason for not interacting with apical P-glycoprotein.

Common substrates for a transporter should show mutual inhibition of each others transport. Ciprofloxacin, at a concentration above its K_m, failed to inhibit the net secretion of vinblastine in Caco-2 cells. The organic anion exchange inhibitor DIDS, which reduces the net secretion of ciprofloxacin, had little effect on the transport and accumulation of vinblastine. Ciprofloxacin was sensitive to inhibition by the P-glycoprotein substrate verapamil. However, in contrast to the effect of verapamil on the P-glycoprotein substrate vinblastine, ciprofloxacin secretion was not abolished except at high and probably toxic concentrations of verapamil. The effect of verapamil on ciprofloxacin uptake was also much smaller compared to that seen with vinblastine. Anti P-glycoprotein antibodies MRK16 and UIC2 which have been shown to be effective inhibitors of P-glycoprotein transport activity (Hunter *et al*., 1993c; Mechetner & Roninson, 1992) and which inhibited vinblastine $J_{b\text{-}a}$ in concurrent measurements, had no effect on the secretory flux of ciprofloxacin. Taken together, these results suggest that the multidrug efflux pump P-glycoprotein does not have a major role in the mediation of ciprofloxacin secretion in Caco-2 epithelia.

These results agree with a study by Rubinstein *et al*. (1994), which showed that nifedipine had no effect on the secretion of ciprofloxacin in rat intestine, suggesting a lack of involvement of P-glycoprotein. Also, in contrast to difloxacin, which increased the sensitivity of the multidrug resistant P388/adriamycin cell line to daunorubicin, adriamycin and vincristine, ciprofloxacin had no chemosensitizing activity (Gupta *et al*., 1995).

A model for ciprofloxacin secretion could involve active transport across the basolateral membrane, followed by leak across the apical membrane, or a basolateral leak followed by active secretion across the apical membrane. The calculated ratio of ciprofloxacin permeabilities at the basolateral membrane ($P_{b\text{-}c}/P_{c\text{-}b}$) was greater than 1, and exceeded that ratio ($P_{a\text{-}c}/P_{c\text{-}a}$) at the apical membrane. This indicates either an active transport step or an additional driving force other than a chemical gradient at the basolateral membrane. An active accumulation at the basolateral membrane is combined with a rate-limiting step diffusive loss at the apical membrane maintaining transepithelial secretion. Such a model is likely to be oversimplified, and ciprofloxacin may access a number of transporters at both membrane faces. Previous studies have shown that ciprofloxacin transport may be differentially inhibited by other fluoroquinolones at either membrane (Griffiths *et al*., 1994).

In the analysis of results the possibility of dividing ciprofloxacin into acidic endomembrane compartments should be considered. This would reduce the free cytosolic component of intracellular ciprofloxacin and could potentially create a driving force for the accumulation of ciprofloxacin across the basolateral membrane, by creating a chemical gradient for the diffusion of ciprofloxacin into the cell. Lysomotrophic agents may diffuse into lysosomes in their uncharged form and then the weak basic form becomes trapped in the lysosomal compartment (Steinburg, 1994). It seems unlikely that intracellular division via non-ionic diffusion of ciprofloxacin could account for accumulative transport at the basolateral membrane, because a decrease in intracellular pH_i did not markedly alter net ciprofloxacin secretion but reduced cellular accumulation from the basal solution.

Carlier *et al*. (1990, 1991) used cell fractionation methods to determine the intracellular location of fluoroquinolones in macrophages. More than 80% of subcellular ciprofloxacin, perfloxacin, lomefloxacin and fleroxacin was present in the cytoplasm, with only a small percentage being associated with endosomes and lysosomes. This contrasts with the cellular localization of azithromycin and roxithromycin. These two antibiotics were partly localized in lysosomes (70% and 50%, respectively). Studies of intracellular localization of Caco-2 cells have not been carried out, but it appears probable that most of the cellular ciprofloxacin is free in the cytoplasm, since ciprofloxacin is released from the cell upon lysis (Griffiths *et al*., unpublished data).

Secretion of ciprofloxacin across Caco-2 cell monolayers was sensitive to inhibition by the organic anion exchange inhibitor 4,4′-diisothiocyanostilbene-2-2′-disulphonic acid (DIDS). The disulphonic stilbenes SITS and DNDS, compounds related to DIDS, had no effect on ciprofloxacin flux. This demonstrates that the anion exchanger is not involved in ciprofloxacin transport in Caco-2 cells. Taken together with other inhibitor data (above) it seems likely that the zwitterion is the transported species. Whilst DIDS is an irreversible covalent modifier, having a reactive thiocyanate group which modifies lysine residues, the effect of DNDS was reversible and it lacks this reactive group (Cabantchik & Rothstein, 1974). Perhaps DIDS causes the membrane transport protein to be modified which DNDS is unable to do.

DIDS only had an inhibitory effect on ciprofloxacin transport when it was present in the basolateral compartment. Apical administration of DIDS had no effect on transport or uptake of DIDS at the basolateral membrane. This, in conjunction with the large increase in cellular uptake of ciprofloxacin suggests that DIDS may access the intracellular compartment from the basolateral surface and have an inhibitory effect from the cytosolic surface reducing the exit permeabilities.

The inhibition of all four unidirectional permeabilities and the differential inhibition of exit permeability suggest there may be a number of transport proteins involved in the transport of ciprofloxacin. Both $P_{c\text{-}a}$ and $P_{c\text{-}b}$ were reduced to a larger degree of $P_{a\text{-}c}$ and $P_{b\text{-}c}$, whilst the asymmetries in permeability at each membrane face increased. The different molar sensitivities at the extracellular and intracellular (cytosolic) compartment demonstrate that the cytosolic surface is more sensitive to DIDS than the extracellular surface. The large asymmetry in permeability ratios suggest that additional driving forces operate to accumulate ciprofloxacin in the cytosol. At present these driving forces have not been identified.

The inhibitory site of action of DIDS on red blood cell Cl^- exchangers is primarily exofacial (Barzilay & Cabantchik, 1979) and it is thought not to permeate the cell membrane. However, a more recent study in the colonic cell line T84 found that basolateral administration of stilbenes stimulated Cl^-

secretion by elevating Ca^{2+}, whilst apical administration had no effect (Brayden *et al*., 1993). The authors suggested that a specific basolateral uptake mechanism existed that allowed DIDS to accumulate within the cytosol from the basal solution. A similar explanation is consistent with the inhibitory effects of DIDS on ciprofloxacin transport.

Verapamil also inhibited net secretion and increased cellular uptake of ciprofloxacin though with a lower potency than the effect it has on P-glycoprotein-mediated vinblastine secretion. Verapamil appeared to block the exit pathway for ciprofloxacin at the apical membrane ($P_{c\text{-}a}$), thus suggesting the involvement of an apical efflux system in the secretion of ciprofloxacin. Verapamil also increased $P_{c\text{-}b}$ i.e. the ciprofloxacin permeability from the cell across the basolateral membrane. In this regard, the effects of DIDS and verapamil were opposite.

For the cationic substrate vinblastine, inhibition of secretion by verapamil is easily reconciled with a potential-dependent uptake and concentration at the basolateral membrane (thus giving the large asymmetry in permeabilities at this membrane) followed by ATP-dependent extrusion at the apical membrane mediated by P-glycoprotein. The major effect of verapamil was to decrease $P_{c\text{-}a}$, consistent with P-glycoprotein inhibition. In the absence of this pumped flux, apical membrane permeability will again be dominated by potential-driven influx.

Fluoroquinolones are effluxed from bacterial cells by multidrug efflux pumps. The eukaryotes *Myocarbacterium smegmatics* (Liu *et al*., 1996), *Escherichia coli* (Cohen *et al*., 1988), *Staphylococcus aureus* (Kaatz *et al*., 1993; Neyfakh *et al*., 1993) and *Bacillus subtilis* (Ahmed *et al*., 1995) all transport fluoroquinolones by an active efflux multidrug specific transport mechanism. Though the current data convincingly argue against the involvement of P-glycoprotein, it is worth noting that P-glycoprotein is a member of the ABC family of transporters, other members expressed in human tissue include the cystic fibrosis transmembrane conductance regulator (Ames, 1992) and multidrug resistance-associated protein (Jedlitschky *et al*., 1994). Members of this transporter family are found throughout the animal kingdom including in bacteria (Ames, 1992). It is thus possible that ciprofloxacin secretion in the human intestinal cell line Caco-2 is mediated by an unidentified multidrug-resistance like mechanism. It is likely that further understanding of the secretory mechanism in human intestinal cells will require molecular characterization of the transporters involved.

M.E.C. was a BBSRC-CASE student with Glaxo Wellcome Research and Development Ltd. [^{14}C]-ciprofloxacin was a generous gift from Dr U. Pleiß (Bayer AG Institut fur Pharmacokinetik, Wuppertal, Germany).

References

AHMED, M., LYASS, L., MARKHAM, P.N., TAYLOR, S.S., VAZQUEZ-LASLOP & NEYFAKH, A.A. (1995). Two highly similar multidrug transporters of *Bacillus subtilis* whose expression is differentially regulated. *J. Bacteriol*., **177,** 3904 – 3910.

AMES, G.F.L. (1992). Bacterial periplasmic permeases as model systems for the superfamily of traffic ATPases, including the multidrug resistance protein and the cystic fibrosis transmembrane conductance regulator. *Int. Rev. Cytol*., **137,** 1 – 35.

AWASTHI, S., SINGHAI, S.S., SRIVASTAVA, S.K., ZIMNIAK, P., BAJPAI, K.K., SAXENA, M., SHARMA, R., ZILLER, S.A., FRENKEL, E.P., SINGH, S.V., NONG, G.H. & AWASTHI, Y.C. (1994). Adenosine triphosphate-dependent transport of doxorubicin, daunomycin and vinblastine in human tissues by a mechanism distinct from the P-glycoprotein. *J. Clin. Invest*., **93,** 958 – 965.

BARKER, G. & SIMMONS, N.L. (1981). Identification of two strains of cultures canine renal epithelial cells (MDCK cells) which display entirely different physiological properties. *Q. J. Exp. Physiol*., **66,** 61 – 72.

BARZILAY, M. & CABANTCHIK, Z.I. (1979). Anion transport in red blood cells. Sites and sidedness of inhibition by high-affinity reversible binding probes. *Membr. Biochem*., **2,** 297 – 322.

BRAYDEN, D., KROUSE, M.E., LAW, T. & WINE, J.J. (1993). Stilbenes stimulate T84 Cl^- secretion by elevation Ca^{2+}. *Am. J. Physiol*., **264,** G325 – G333.

CABANTCHIK, Z. & ROTHSTEIN, A. (1974). Membrane proteins related to anion permeability of human red blood cells. I. Localisation of disulfonic stilbene binding sites in proteins involved in permeation. *J. Membr. Biol*., **15,** 207 – 226.

CAO, C.X., SILVERSTEIN, S.C., NEU, H.C. & STEINBERG, T.H. (1992). J774 macrophages secrete antibiotics via organic anion transporters. *J. Infect Dis*., **165,** 322 – 328.

CARLIER, M.B., SCORNEAUX, B. & TULKENS, P.M. (1991). Accumulation, subcellular distribution and activity of azithromycin (Az) compared to roxithromycin (Rx) and ciprofloxacin (Cp) in J774 macrophages (MO). In *Abstracts, 31st Interscience Conference on Antimicrobial Agents and Chemotherapy*. New York: American Society of Microbiology.

CARLIER, M.B., SCORNEAUX, B., ZENEBERGH, A., DESNOTTES, J. & TULKENS, P.M. (1990). Cellular uptake, localisation and activity of fluoroquinolones in uninfected and infected macrophages. *J. Antimicrobial. Chemother*., **26** (B), 27 – 39.

CAVET, M.E. (1996). *Intestinal Secretion of Organic Solutes*, pp. 109 – 114. Ph.D. Thesis, University of Newcastle upon Tyne.

CAVET, M.E., WEST, M. & SIMMONS, N.L. (1996). Transport and epithelial secretion of the cardiac glycoside by human intestinal epithelial Caco-2 cells. *Br. J. Pharmacol*., **118,** 1389 – 1396.

COHEN, S., HOOPER, D.C., WOLFSON, J.S., SOUZA, K.S., MCMURRY, L.M. & LEVY, S.B. (1988). Endogenous active efflux of norfloxacin in susceptible *Escherichia coli*. *Antimicrobial Agents Chemother.,* **32,** 1187 – 1191.

ELFERINK, P.J.O., BAKKER, C.T.M. & JANSEN, P.L.M. (1993). Glutathione-conjugate transport by human colon adenocarcinoma cells (Caco-2 cells). *Biochem. J*., **290,** 759 – 764.

EVERS, R., ZAMAN, C.G.R., VAN DEETMER, L., JANSEN, H., CALAFAT, J., OOMEN, L.C.J.M., ELFERINK, P.J.O., BORST, P. & SCHINKEL, A.H. (1996). Basolateral localisation and export activity of the human multidrug resistance-associated protein in polarised pig kidney cells. *J. Clin. Invest*., **97,** 1211 – 1218.

FLENS, M., ZAMAN, G.J.R., VAN DER VALK, P., IZQUIERDO, M.A., SCHROEIJERS, A.B., SCHEFFER, G.L., VAN DER GROEP, P., HAAS, M., MEIJER, C.J.L.M. & CHEPER, R.J. (1996). Tissue distribution of the multidrug resistance protein. *Am. J. Pathol*., **148,** 1237 – 1247.

GRANT, C., VALDIMARSSO, G., HIPFNER, D.R., ALMQUIST, K.C., COLE, S.P.C. & DEELEY, R.G. (1994). Overexpression of multidrug resistance associated protein (MRP) increases resistance to natural products. *Cancer Res*., **54,** 357 – 361.

GRIFFITHS, N.M., HIRST, B.H. & SIMMONS, N.L. (1993). Active secretion of the fluoroquinolone ciprofloxacin by human intestinal epithelial Caco-2 cell layers. *Br. J. Pharmacol*., **108,** 575 – 576.

GRIFFITHS, N.M., HIRST, B.H. & SIMMONS, N.L. (1994). Active intestinal secretion of the fluoroquinolone antibacterials ciprofloxacin, norfloxacin and perfloxacin; a common secretory pathway? *J. Pharmacol. Exp. Ther*., **269,** 496 – 502.

GUPTA, S., THADEPALLI, F. & GOLLAPUDI, S. (1995). Difloxacin reverses multidrug resistance in P388/ADR cells via a mechanism independent of P-glycoprotein and without correcting drug transport or subcellular drug distribution. *Int. J. Oncol*., **7,** 475 – 480.

HIRANO, T., ISEKI, K., SATO, I., MIYAZAKI, S., TAKADA, M., KOBAYASHI, M., SUGAWARA, M. & MIYAZAKI, S. (1995a). The intestinal transport mechanism of fluoroquinolones: Inhibitory effect of ciprofloxacin, an enoxacin derivative, on the membrane potential-dependent uptake of enoxacin. *Pharmacol. Res*., **12,** 1299 – 1303.

HIRANO, T., ISEKI, K., SUGAWARA, M., MIYAZAKI, S., TAKADA, M. & MIYAZAKI, K. (1995b) Transport mechanism of enoxacin in rat brush-border membrane of renal cortex: Interaction with organic cation transport system and ionic diffusion potential dependent uptake. *Biol. Pharmac. Bull*., **18,** 342 – 346.

HORIO, M., CHIN, K.V., CURRIER, S.J., GOLDENBERG, S., WILLIAMS, C., PASTAN, I., GOTTESMAN, M.M. & HANDLER, J. (1989). Transepithelial transport of drugs by the multidrug transporter in cultured Madin-Darby canine kidney cell epithelia. *J. Biol. Chem.*, **264,** 14880 – 14884.

HUNTER, J., HIRST, B.H. & SIMMONS, N.L. (1993a). Drug absorption limited by P-glycoprotein-mediated secretory drug transport in human intestinal epithelial Caco-2 cell layers. *Pharmacol. Res.*, **10,** 743 – 749.

HUNTER, J., HIRST, B.H. & SIMMONS, N.L. (1993b). Transepithelial secretion, cellular accumulation and cytotoxicity of vinblastine in defined MDCK cell strains. *Biochim. Biophys. Acta*, **1179,** 1 – 10.

HUNTER, J., JEPSON, M.A., TSURUO, T., SIMMONS, N.L. & HIRST, B.H. (1993c). Functional expression of P-glycoprotein in apical membranes of human intestinal Caco-2 cells. Kinetics of vinblastine secretion and interaction with modulators. *J. Biol. Chem.*, **268,** 14991 – 14997.

ISEKI, K., HIRANO, T., FUKUSHI, Y., KITAMURA, Y., MIYAZAKI, S., TAKADA, M., SUGARAWA, M., SAITOH, H. & MIYAZAKI, K. (1992). The pH-dependent uptake of enoxacin by rat intestinal brush-border membrane vesicles. *J. Pharm. Pharmacol.*, **44,** 722 – 726.

JACQUEMIN, E., HAGENBUCH, B., STEIGER, B., WOLKOFF, A.W. & MEIER, P.J. (1991). Expression of the hepatocellular chloride-dependent sulfobromophthalein uptake system in xenopus laevis oocytes. *J. Clin. Invest.*, **88,** 2146 – 2149.

JACQUEMIN, E., HAGENBUCH, B., STEIGER, B., WOLKOFF, A.W. & MEIER, P.J. (1994). Expression of a rat liver Na-independent organic anion transporter. *Proc. Natl. Acad. Sci. U.S.A.*, **91,** 133 – 137.

JAEHDE, U., SORGEL, K., NABER, K.G., REITER, A., SEELMANN, R., SIGL, G., MUTH, P. & SCHUNACK, W. (1989). Gastrointestinal secretion of ciprofloxacin (CIP) in healthy volunteers. In *Program and Abstracts of the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Abstract No. 202. Washington, DC: American Society for Microbiology.

JEDLITSCHKY, G., LEIER, I., BUCHHOLZ, U., CENTRE, M. & KEPPLER, D. (1994). ATP-dependent transport of glutathione-S-conjugates by the multidrug resistance-associated protein. *Cancer Res.*, **56,** 988 – 994.

KAATZ, G., SEO, S.M. & RUBLE, C.A. (1993). Efflux-mediated fluoroquinolone resistance in *Staphylococcus aureus*. *Antimicrobial Agents Chemother.*, **37,** 1086 – 1094.

KARLSSON, J., KUO, S.M., ZIEMNIAK, J. & ARTURSSON, P. (1993). Transport of celiprolol across human intestinal epithelial (Caco-2) cells: mediation of secretion by multiple transporters including P-glycoprotein. *Br. J. Pharmacol.*, **110,** 1009 – 1016.

LAUTERBACH, F. (1977a). Fluxes of ions and drugs in isolated guinea-pig intestinal mucosa. *J. Physiol.*, **266,** 27P – 28P.

LAUTERBACH, F. (1977b). Intestinal secretion of organic ions and drugs. In *Intestinal Permeation*. ed. Kramer, M. & Lauterbach, F. pp. 173 – 194. Amsterdam, Oxford: Excerpta Medica.

LAUTERBACH, F. (1983). Intestinal secretion of drugs. In *Intestinale Transport*. ed. Gilles-Baillien M. & Gilles, R. pp. 76 – 86. Berlin, Heidelburg: Springer-Verlag.

LIU, J., TAKIFF, H.E. & NIKAIDO, H. (1996). Active efflux of fluoroquinolones in *Mycobacterium smegmatis* mediated by LfrA, a multidrug efflux pump. *J. Bacteriol.*, **178,** 3791 – 3795.

MAYER, U., WAGENAAR, E., BEIJNEN, J.H., SMIT, J.W., MEIJER, D.K.F., VAN ASPEREN, J., BORST, P. & SCHINKEL, A.H. (1996). Substantial excretion of digoxin via the intestinal mucosa and prevention of long-term digoxin accumulation in the brain by the mdr1a P-glycoprotein. *Br. J. Pharmacol.*, **119,** 1038 – 1044.

MCEWAN, G.T.A., HUNTER, J., HIRST, B.H. & SIMMONS, N.L. (1992). Volume-activated Cl secretion and transepithelial vinblastine secretion mediated by P-glycoprotein are not correlated in cultured human T84 intestinal epithelial layers. *FEBS Lett.*, **304,** 233 – 236.

MECHETNER, E. & RONINSON, I.B. (1992). Efficient inhibition of P-glycoprotein-mediated multidrug resistance with a monoclonal antibody. *Proc. Natl. Acad. Sci.*, **89,** 5824 – 5828.

MIYAMOTO, Y., GANAPATHY, V. & LEIBACH, F.H. (1988). Transport of guanidine in rabbit intestinal brush-border membrane vesicles. *Am. J. Physiol.*, **255,** G85 – G92.

NAFTALIN, R. & CURRAN, P.F. (1974). Galactose transport in rabbit ileum. *J. Membr. Biol.*, **16,** 257 – 278.

NEYFAKH, A., BORSCH, C.M. & KAATZ, G.W. (1993). Fluoroquinolone resistance protein norA of *Staphylococcus aureus* is a multidrug efflux transporter. *Antimicrobial Agents Chemother.*, **37,** 128 – 129.

OKANO, T., MAEGAWA, H., INUI, K.I. & HORI, R. (1990). Interaction of ofloxacin with organic cation transport system in rat renal brush-border membranes. *J. Pharmacol. Exp. Ther.*, **255,** 1033 – 1037.

OOIE, T., SUZUKI, H., TERASAKI, T. & SUGIYAMA, Y. (1996). Characterisation of the transport properties of a quinolone antibiotic, fleroxacin, in rat choroid plexus. *Pharmacol. Res.*, **13,** 523 – 527.

PARRY, M.F., SMEGO, D.A. & DIGIOVANNI, M.A. (1988). Hepatobiliary kinetics and excretion of ciprofloxacin. *Antimicrobial Agents Chemother.*, **32,** 982 – 985.

PETERS, W.H.M. & ROELOFS, H.M.J. (1992). Biochemical characterization of resistance to mitoxanthrone and adriamycin in Caco-2 human adenocarcinoma cells; a possible role for glutathione-S-transferases. *Cancer Res.*, **52,** 1886 – 1890.

PRIETO, J.G., BARRIO, J.P., ALVAREZ, A.I. & GOMEZ, G. (1988). Kinetic mechanism for the intestinal absorption of ofloxacin. *J. Pharm. Pharmacol.*, **40,** 211 – 212.

PRITCHARD, J.B. & MILLER, D. (1993). Mechanism mediating renal secretion of organic anions and organic cations. *Physiol. Rev.*, **73,** 765 – 797.

RABBAA, L., DAUTREY, S., COLAS-LINHART, N., CARBON, C. & FARINOTTI, R. (1995). Stereoselectivity of ofloxacin intestinal transport in the rat. *Drugs*, **49 (Suppl.2)**, 333 – 334.

RAMON, J., DAUTREY, S., FARINOTTI, R., CARBON, C. & RUBINSTEIN, E. (1994). Intestinal elimination of ciprofloxacin in rabbits. *Antimicrobial Agents Chemother.*, **38,** 757 – 760.

ROHWEDDER, R., BERGAN, T., THORSTEINSSON, S.B. & SCHOLL, H. (1990). Transintestinal elimination of ciprofloxacin. *Chemotherapy*, **36,** 77 – 84.

RUBINSTEIN, E., DAUTREY, S., FARINOTTI, R., ST JULIEN, L., RAMON, J. & CARBON, C. (1995). Intestinal elimination of sparfloxacin, fleroxacin, and ciprofloxacin in rats. *Antimicrobial Agents Chemother.*, **39,** 99 – 102.

RUBINSTEIN, E., ST JULIEN, L., RAMON, J., DAUTREY, S., FARINOTTI, R., HUNEAU, J.F. & CARBON, C. (1994). The intestinal elimination of ciprofloxacin in the rat. *J. Infect. Dis.*, **169,** 218 – 221.

SAITOH, H., GERARD, C. & AUNGST, B.J. (1996). The secretory intestinal transport of some beta-lactam antibiotics and anionic compound: A mechanism contributing to poor oral absorption. *J. Pharmacol. Exp. Ther.*, **278,** 205 – 211.

SORGEL, F. & KINZIG, M. (1993a). Pharmacokinetics of gyrase inhibitors, Part 1: Basic chemistry and gastrointestinal disposition. *Am. J. Med.*, **94,** 44S – 55S.

SORGEL, F. & KINZIG, M. (1993b). Pharmacokinetics of gyrase inhibitors, Part 2: Renal and hepatic elimination pathways and drug interactions. *Am. J. Med.*, **94,** 56S – 69S.

SORGEL, F., NABER, K.G., JAEHDE, U., REITTER, A., SEELMAN, R. & SIGL, G. (1989). Brief report: gastrointestinal secretion of ciprofloxacin. Evaluation of the charcoal model for investigations in healthy volunteers. *Am. J. Med.*, **87,** 62S – 65S.

SORGEL, F., NABER, K.G., KINZIG, M., MAHR, G. & MUTH, P. (1991). Comparative pharmacokinetics of ciprofloxacin and temafloxacin in humans: A review. *Am. J. Med.*, **91,** 51S – 65S.

STEINBURG, T. (1994). Cellular transport of drugs. *Clin. Infect. Dis.*, **19,** 916 – 921.

THWAITES, D.T., BROWN, C.D.A., HIRST, B.H. & SIMMONS, N.L. (1993a). Transepithelial glycylsarcosine transport in intestinal Caco-2 cells mediated by expression of H^+-coupled carriers at both apical and basal membranes. *J. Biol. Chem.*, **268,** 7640 – 7642.

THWAITES, D.T., BROWN, C.D.A., HIRST, B.H. & SIMMONS, N.L. (1993b). H^+-coupled dipeptide (glycylsarcosine) transport across apical and basal borders of human intestinal Caco-2 cell monolayer display distinctive characteristics. *Biochem. Biophys. Acta*, **1151,** 237 – 245.

THWAITES, D.T., HIRST, B.H. & SIMMONS, N.L. (1994). Substrate specificity of the di/tripeptide transporter in human intestinal epithelia (caco-2): identification of substrates that undergo H^+-coupled absorption. *Br. J. Pharmacol.*, **113,** 1050 – 1056.

THWAITES, D.T. & SIMMONS, N.L. (1996). pH sensitivity of amino-acid transport at the brush-border of human intestinal epithelial (Caco-2) cells is consistent with H^+ symport. *J. Physiol.*, **495,** 96P.

TURNHEIM, K. & LAUTERBACH, F. (1972). Intestinal transport of quarternary ammonium compounds in vivo. *Naunyn-Schmiedeberg's Achiv. Pharmacol.* **274,** R118.

TURNHEIM, K. & LAUTERBACH, F. (1977). Absorption and secretion of monoquarternary ammonium compounds by the isolated intestinal mucosa. *Biochem. Pharmacol*., **26,** 99–108.

TURNHEIM, K. & LAUTERBACH, F. (1980). Interaction between intestinal absorption and secretion of monoquarternary ammonium compounds in guinea pigs - A concept of the absorption kinetics of organic cations. *J. Pharmacol. Exp. Ther*., **212,** 418–424.

ULLRICH, K.J., RUMRICH, G., DAVID, C. & FRITZSCH, G. (1993a). Bisubstrates: Substances that interact with renal contraluminal organic anion and organic cation transport systems. II. Zwitterionic substrates: dipeptides, cephalosporins, quinolonecarboxylate gyrase inhibitors and phosphamide thiazine carboxylates; Nonionizable substrates: Steroid hormones and cyclophosphamides. *Pflügers Archiv. Eur. J. Physiol*., **425,** 300–312.

ULLRICH, K.J., RUMRICH, G., DAVID, C. & FRITZSCH, G. (1993b). Bisubstrates: Substances that interact with renal contraluminal organic anion and organic cation transport systems. I. Amines, piperidines, piperazines, azepines, pyridine, quinolines, imidazoles, thiazoles, guanidines and hydrazines. *Pflügers Archiv. Eur. J. Physiol*., **425,** 280–299.

ULLRICH, K.J. & RUMRICH, G. (1988). Contraluminal transport systems in the proximal tubule involved in secretion of organic anions. *Am. J. Physiol*., **254,** F453–F462.

WOLKOFF, A., SAMUELSON, A.C., JOHANSEN, K.L., NAKATA, R., WITHERS, D.M. & SOSIAK, A. (1987). Influence of Cl^- on organic anion transport in short-term cultured rat hepatocytes and isolated perfused rat liver. *J. Clin. Invest*., **79,** 1259–1268.

(Received December 18, 1996
Revised April 10, 1997
Accepted May 7, 1997)