Active secretion of the fluoroquinolone ciprofloxacin by human intestinal epithelial Caco-2 cell layers

¹Nina M. Griffiths, Barry H. Hirst & Nicholas L. Simmons

Gastrointestinal Drug Delivery Research Centre, and Department of Physiological Sciences, Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, NE2 4HH

The bidirectional transepithelial fluxes of ciprofloxacin, an antibacterial fluoroquinolone, across the human intestinal epithelial Caco-2 cell-line show marked asymmetry. Basal-to-apical flux of ciprofloxacin (10 μM) exceeds apical-to-basal flux indicating net secretion. Net ciprofloxacin secretion is abolished by azide/2-deoxy-D-glucose treatment, displays saturation kinetics ($K_m = 0.89 \pm 0.23 \text{ mM}$, $V_{\text{max}} = 44.3 \pm 4.9 \text{ nmol cm}^{-2}\text{h}^{-1}$) and competition by other fluoroquinolones. A specific, active secretion in Caco-2 epithelia may explain the transintestinal elimination of ciprofloxacin observed in pharmacokinetic studies in man.

Keywords: Caco-2 cells; ciprofloxacin; epithelia; fluoroquinolone; intestinal secretion

Introduction In human volunteers, 18% of an intravenous dose of ciprofloxacin, an antibacterial fluoroquinolone, is eliminated by a non-renal process across the intestine (Sorgel *et al.*, 1989; 1991). In renal failure patients, transintestinal ciprofloxacin elimination was increased to approximately 40% of an intravenous dose (Rohwedder *et al.*, 1990). Since biliary elimination of the drug is small and only a minor component of ciprofloxacin in faeces is metabolites (Sorgel *et al.*, 1989; Rohwedder *et al.*, 1990) it is apparent that an unidentified intestinal secretory mechanism for this drug must exist.

The Caco-2 cell-line is being used increasingly as an *in vitro* model for absorption studies (Hidalgo *et al.*, 1989). This human colonic cell-line expresses a number of brush-border membrane hydrolases and membrane transport systems consistent with a predominantly small intestinal phenotype (Inui *et al.*, 1992). Here we report that ciprofloxacin undergoes substantial active secretion in Caco-2 epithelial layers. The relationship between ciprofloxacin secretion and other identified transport systems in Caco-2 cells is discussed.

Methods *Cell culture* Caco-2 cells (85–100 serial passages) were obtained from Dr I. Hassan (Ciba-Geigy Pharmaceuticals, Horsham, Sussex) and grown in Dulbecco's modified Eagle's medium (DMEM) containing D-glucose (4.5 g l⁻¹) supplemented with foetal calf serum (10%), glutamine (2 mM), non-essential amino acids (1%) and gentamycin (50 $\mu\text{g ml}^{-1}$). Epithelial layers were obtained by high-density seeding (3×10^6 cells) onto permeable matrices (Anocells, Nunc 25 mm culture inserts) and used in experiments after 11 days.

Transepithelial [¹⁴C]-ciprofloxacin fluxes: Cell monolayers were washed in Krebs buffer (composition all mmol l⁻¹) NaCl 137, KCl 5.4, CaCl₂ 2.8, MgSO₄ 1.2, NaH₂PO₄ 0.3, KH₂PO₄ 0.3, Tris base 14, HCl 12, glucose 5 (pH 7.4 at 37°C), placed in fresh 6-well plates containing 2 ml of Krebs (basal solution). Krebs solution (2 ml) was added to the apical side and [¹⁴C]-ciprofloxacin (10 μM at 61.24 $\mu\text{Ci mg}^{-1}$, or with increasing concentrations of ciprofloxacin) was present in either apical or basal solution. Unlabelled ciprofloxacin was included in the contralateral solution. Bidirectional fluxes (apical-to-basal, J_{a-b} ; basal-to-apical J_{b-a}) of ciprofloxacin were measured at 1 h time intervals for up to 3 h (200 μl samples) with at least three replicates for each condition. Cell-associated radioactivity was determined following

extensive washing of culture inserts (4 changes of 1 l buffer at 4°C) and removal of filters to counting vials. As an indicator of monolayer integrity, [³H]-mannitol fluxes were determined simultaneously with [¹⁴C]-ciprofloxacin. ¹⁴C- and ³H-activities were determined by scintillation counting, appropriate corrections being made for quench and cross-channel spill-over.

Materials [¹⁴C]-ciprofloxacin (61.24 $\mu\text{Ci mg}^{-1}$) and unlabelled ciprofloxacin were generous gifts of Bayer (Wuppertal, Germany). [³H]-mannitol (30 Ci mmol⁻¹) was from Amersham International plc (Little Chalfont, Amersham, Bucks). Scintillation cocktail 'Optiphase safe' was from Pharmacia LKB Biotechnology Ltd (Milton Keynes, Bucks). All tissue culture media and reagents (Gibco BRL), tissue culture plastics and tissue culture inserts (Nunc) were supplied by Life Technologies Ltd (Paisley, Scotland). All other reagents/chemicals were obtained from Sigma Chemical Co. (Poole, Dorset) or BDH Chemicals Ltd (Poole, Dorset).

Statistics Flux rates (pmol cm⁻²h) were obtained by linear regression of time courses (each hour over 1–3 h) $\pm$ s.e., where d.f. denotes the degree of freedom (3–6 monolayers per condition). Net ciprofloxacin flux, $J_{\text{net}} = J_{b-a} - J_{a-b}$.

Results The bidirectional transepithelial fluxes for ciprofloxacin (10 μM) were linear up to 3 h. Basal-to-apical flux (J_{b-a} , $451 \pm 52.5 \text{ pmol cm}^{-2}\text{h}^{-1}$, d.f. = 22) was greater than apical-to-basal flux (J_{a-b} , $126 \pm 10 \text{ pmol cm}^{-2}\text{h}^{-1}$, d.f. = 22) indicating a substantial net secretion of ciprofloxacin from basal to apical surfaces (J_{net} , $325 \text{ pmol cm}^{-2}\text{h}^{-1}$). Simultaneous measurements of bidirectional mannitol fluxes showed no marked asymmetry (J_{a-b} and J_{b-a} were 52.3 and 47.5 fmol cm⁻²h, respectively). In order to test the dependence of ciprofloxacin fluxes upon cellular ATP, fluxes were determined in the presence of 15 mM Na azide and 50 mM 2-deoxy-D-glucose in both apical and basal solutions. The substantial net flux observed in control epithelial layers (325 pmol cm⁻²h) was virtually eliminated (to 27 pmol cm⁻²h) both by a reduction in J_{b-a} from 434 ± 55 (d.f. = 10) to 233 ± 28 (d.f. = 10) pmol cm⁻²h and an increment in J_{a-b} from 104 ± 11 (d.f. = 10) to 214 ± 40 (d.f. = 10) pmol cm⁻²h. The increment in J_{a-b} is likely to result from an increased paracellular flux as mannitol flux is increased concurrently (from control J_{a-b} of 36.3 ± 11.6 to $85.3 \pm 12.6 \text{ fmol cm}^{-2}\text{h}^{-1}$ in azide/2-deoxy-D-glucose).

Figure 1 shows the concentration-dependence of the bidirectional ciprofloxacin fluxes. Whereas J_{a-b} is a simple linear function of ciprofloxacin concentration, J_{b-a} is non-linear. J_{net}

¹ Author for correspondence.

Figure 1 Concentration-dependence of [^{14}C]-ciprofloxacin fluxes; (■) J_{a-b} (apical-to-basal flux), (▲) J_{b-a} (basal-to-apical flux), (◇) J_{net} (net flux = $J_{b-a} - J_{a-b}$). Solid line for J_{a-b} is the linear regression line for the data $y = 9.1x + 0.44$, $r = 0.99$, d.f. = 22, $P < 0.001$. Solid line for J_{net} was calculated by non-linear regression assuming Michaelis-Menten kinetic fit for the data; $K_m = 0.89 \pm 0.23$ nM, $V_{\text{max}} = 44.3 \pm 4.9$ nmol cm^{-2} .h.

demonstrates saturation kinetics with half-maximal saturation being observed at 0.89 ± 0.23 mM (d.f. = 22) and a maximal secretory capacity of 44.3 ± 4.9 nmol cm^{-2} .h. Solubility of ciprofloxacin limited the use of higher concentrations.

Competitive inhibition of $10 \mu\text{M}$ [^{14}C]-ciprofloxacin net flux (J_{net}) was observed by other unlabelled fluoroquinolones added to the same solution as the tracer [^{14}C]-ciprofloxacin: J_{net} calculated at 3 h (n values = paired cell layers), control ($10 \mu\text{M}$ ciprofloxacin) 255 ± 34 pmol cm^{-2} .h ($n = 10$); with 1 mM flumequine 175 ± 10 pmol cm^{-2} .h (32% inhibition; $n = 6$); with 1 mM enoxacin 162 ± 15 pmol cm^{-2} .h (36% inhibition; $n = 6$); with 1 mM norfloxacin 227 ± 2 pmol cm^{-2} .h (11% inhibition; $n = 5$). Verapamil ($100 \mu\text{M}$) reduced [^{14}C]-ciprofloxacin net secretion by 38% compared to control, from 152 ± 10 to 94 ± 11 pmol cm^{-2} .h ($n = 3$). Indomethacin ($100 \mu\text{M}$) was without effect on [^{14}C]-ciprofloxacin transport; net secretory flux 378 ± 12 and 392 ± 32 pmol cm^{-2} .h ($n = 3$) with and without indomethacin, respectively.

References

- COLLINGTON, G.K., HUNTER, J., ALLEN, C.N., SIMMONS, N.L. & HIRST, B.H. (1992). Polarised efflux of BCECF from cultured epithelial cell monolayers. *Biochem. Pharmacol.*, **44**, 417–424.
- HIDALGO, I.J., RAUB, T.J. & BORCHARDT, R.T. (1989). Characterisation of the human colon carcinoma cell-line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology*, **96**, 734–749.
- HUNTER, J., HIRST, B.H. & SIMMONS, N.L. (1991a). Epithelial secretion of vinblastine by human intestinal adenocarcinoma cell (HCT-8 and T84) layers expressing P-glycoprotein. *Br. J. Cancer*, **64**, 437–444.
- HUNTER, J., HIRST, B.H. & SIMMONS, N.L. (1991b). Transepithelial vinblastine secretion mediated by P-glycoprotein is inhibited by forskolin derivatives. *Biochem. Biophys. Res. Commun.*, **181**, 671–676.
- INUI, K.I., YAMAMOTO, M. & SAITO, H. (1992). Transepithelial transport of oral cephalosporins by monolayers of intestinal cell-line Caco-2: specific transport systems in the apical and basolateral membranes. *J. Pharmacol. Exp. Ther.*, **261**, 195–201.
- ISRAILI, Z.H. & DAYTON, P.G. (1984). Enhancement of xenobiotic elimination: role of intestinal excretion. *Drug Metab. Rev.*, **15**, 1123–1159.
- ROHWEDDER, R., BERGAN, T., THORSTEINSSON, S.B. & SCHOLL, H. (1990). Transintestinal elimination of ciprofloxacin. *Chemotherapy*, **36**, 77–84.
- SORGEL, F., NABER, K.G., JAEHDE, U., REITER, A., SEELMAN, R. & SIGL, G. (1989). Gastrointestinal secretion of ciprofloxacin. *Am. J. Med.*, **87**, 63–65S.
- SORGEL, F., NABER, K.G., MAHR, G. & MUTH, P. (1991). Comparative pharmacokinetics of ciprofloxacin and temafloxacin in humans: a review. *Am. J. Med.*, **91**, 51–68S.

(Received November 23, 1992
Accepted December 9, 1992)

Discussion The present data show that Caco-2 cells possess a specific mechanism for the transepithelial secretion of ciprofloxacin from basolateral to apical bathing media. Active transport is indicated since this net ciprofloxacin secretion is generated in the absence of an external driving force and is abolished by azide/2-deoxy-D-glucose implying a dependence upon cellular metabolism. Ciprofloxacin secretion exhibits saturation kinetics and is subject to competitive inhibition by compounds of a similar structure. That ciprofloxacin secretion is observed in human intestinal cells in culture suggests that this system may be useful in elucidating the exact nature of transintestinal elimination of this class of antibacterials.

Transintestinal elimination as a route for xenobiotic clearance has been noted previously for a diverse range of substances including cardiac glycosides, quinine and erythromycin (Israili & Dayton, 1984). Recently it has been suggested that functional expression of P-glycoprotein within the epithelial cells of the intestinal tract may mediate the elimination (from plasma to the intestinal lumen) of numerous drugs (e.g., vinblastine) shown to be substrates for this ATP-dependent pump (Hunter *et al.*, 1991a). Transepithelial vinblastine secretion in a variety of cell lines is virtually abolished by verapamil at $100 \mu\text{M}$, with an IC_{50} of around $2\text{--}20 \mu\text{M}$ (Hunter *et al.*, 1991a,b). The incomplete inhibition of ciprofloxacin secretion by $100 \mu\text{M}$ verapamil may suggest the lack of involvement of P-glycoprotein. In addition recent work has demonstrated that the anionic fluorochrome BCECF may also be the subject of ATP-dependent export across the apical (luminal border) of Caco-2 cells mediated by an indomethacin-sensitive mechanism distinct from P-glycoprotein (Collington *et al.*, 1992). The absence of indomethacin inhibition suggests that the transporters involved in ciprofloxacin secretion are distinct from those mediating BCECF export.

Ciprofloxacin secretion is thus mediated by an active transport system. Whether this represents a novel active secretion system of an as yet unidentified physiological substrate remains to be elucidated.

This work was supported under the LINK Programme in Selective Drug Delivery and Targeting, funded by SERC/MRC/DTI and Industry (SERC Grant GR/F 09747). Charlotte Ward and Maxine Geggie provided expert technical assistance. [^{14}C]-ciprofloxacin was a generous gift from Drs H.P. Krause and U. Pleiß, Bayer AG Institut für Pharmacokinetik, Wuppertal, Germany.