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Active Secretion of Drugs from the Small Intestinal Epithelium in Rats by P-Glycoprotein Functioning as an Absorption Barrier

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Abstract

Because the significance of P-glycoprotein in the in-vivo secretion of β-blockers in intestinal epithelial cells is unclear, the secretory mechanism for β-blockers and other drugs has been evaluated.

Uptake of the β-blockers acebutolol, celiprolol, nadolol and timolol, and the antiarrhythmic agent, quinidine by the multidrug-resistant leukaemic cell line variant K562/ADM was significantly lower than that by drug-sensitive K562 cells, suggesting that these β-blockers are transported by P-glycoprotein out of cells. The reduced uptake of acebutolol by the drug-resistant K562/ADM cells was reversed by treating the cells with anti-P-glycoprotein monoclonal antibody, MRK16, whereas no such alteration in uptake was observed for drug-sensitive K562 cells. Acebutolol uptake by K562/ADM cells was, moreover, markedly enhanced, in a concentration-dependent manner, in the presence of the specific P-glycoprotein inhibitors, MS-209 and cyclosporin. Caco-2 cells were used for evaluation of the role of P-glycoprotein in intestinal permeability to drugs in-vitro. Basolateral-to-apical transport of acebutolol was twice that in the reverse direction. A similar polarized flux was also observed in the transport of vinblastine, but not in that of acetamide or mannitol. When in-vivo intestinal absorption was evaluated by the rat jejunal loop method, with simultaneous intravenous administration of a P-glycoprotein inhibitor, cyclosporin, intestinal absorption of both acebutolol and vinblastine increased 2·6- and 2·2-fold, respectively, but no such enhancement was observed in the absorption of acetamide. The effect of cyclosporin on the intestinal absorption of several drugs was further examined, and the extent of the contribution of P-glycoprotein as an absorption barrier to those drugs was evaluated. ATP depletion by occlusion of the superior mesenteric artery resulted in a clear increase in epithelial permeability to vinblastine, but not to 3-*O*-methylglucose or acetamide, indicating that vinblastine is secreted by ATP-dependent P-glycoprotein into the lumen.

These findings demonstrate that P-glycoprotein plays a role as an absorption barrier by transporting several drugs from intestinal cells into the lumen.

P-Glycoprotein has been found to be a major cause of multi-drug-resistance of tumour cells because it reduces the accumulation of the anticancer drugs by pumping them out of cells (Beck 1987). Functionally P-glycoprotein is characterized by surprisingly broad substrate specificity, including anticancer drugs, calcium channel blockers, immunosuppressive agents and others, and by ATP-dependent primary active transport as the ABC (ATP-binding cassette) transporter superfamily (Hyde et al 1990). P-Glycoprotein has also been shown to be present and function as a transporter in the plasma membranes of many normal tissues (Thiebaut et al 1987; Tsuji et al 1992, 1993; Watanabe et al 1992; Hori et al 1993; Gatmaitan & Arias 1993; Sakata et al 1994; Ohnishi et al 1995). The functional significance of P-glycoprotein as a drug efflux pump in certain normal tissues was clearly demonstrated by generating a 'knock-out mouse' that was disrupted with P-glycoprotein encoded by mdr1a gene (Shinkel et al 1994). In mice lacking a mdr1a gene product, distribution of an anticancer drug, vinblastine, and an insecticide, ivermectin, was enhanced in many tissues especially in the brain. Intestinal distribution of these drugs was also increased in those mice, which is consistent with previous findings that P-glycoprotein is localized on the luminal membrane of intestinal epithelial cells and transports certain drugs, including peptides, out of the cells into the lumen (Hunter et al 1991, 1993; Ince et al 1991; Meyers et al 1991; Spoelstra et al 1991; Hsing et al 1992; Augustijins et al 1993; Burton et al 1993; Wils et al 1994; Zacherl et al 1994; Leu & Huang 1995; Phung-Ba et al 1995).

β-Adrenoceptor antagonists (β-blockers), have been reported to differ in their ability to be absorbed by the intestine depending partly on their lipophilicity. On the basis of correlations between intestinal permeabilities and lipid solubilities, however, some β-blockers, e.g. acebutolol, have unexpectedly low permeability across the intestinal epithelial cells (Taylor et al 1985). One β-blocker, celiprolol has been shown to be transported into the intestinal lumen directly from the blood (Kuo et al 1994); the secretory mechanisms were ascribed to specific transporters including P-glycoprotein or organic cation transporter, or both, by using an intestinal epithelial cell line, Caco-2 (Karlsson et al 1993). The extent of the contribution of P-glycoprotein-mediated secretion of β-blockers in-vivo, however, remains to be clarified.

In this study the significance of P-glycoprotein as an intestinal epithelial secretory mechanism of β-blockers, using mainly acebutolol and typical drugs involved in the multidrug-resistance phenotype, was evaluated by using the intestinal epithelial cultured cell line Caco-2, in-vivo rat intestinal tissue preparations, and a multidrug-resistant cancer cell line, K562/ADM, that overexpresses P-glycoprotein (Tsuruo et al 1986).

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Materials and Methods

Materials

[^{3}H]Vinblastine sulphate (11 Ci mmol^{-1}) and [^{3}H]cyclosporin (7·5 Ci mmol^{-1}) were purchased from Amersham International (Buckinghamshire, UK). [^{3}H]Quinidine (15 Ci mmol^{-1}) and [^{14}C]acetamide (55 mCi mmol^{-1}) were from ARC (St Louis, MO, USA). [^{3}H]PEG900 (5 mCi g^{-1}), [^{3}H]mannitol (26·4 Ci mmol^{-1}) and [^{3}H]3-*O*-methylglucose (87·6 Ci mmol^{-1}) were from New England Nuclear (Boston, MA, USA). Monoclonal antibody MRK16 was purchased from Kyowa Medics (Tokyo, Japan). Unlabelled cyclosporin and MS-209 were kindly supplied by Sandoz AG (Basel, Switzerland) and Mitsui Pharmaceutical Industries Ltd (Tokyo, Japan), respectively. All other chemicals were of reagent grade and were obtained commercially.

Cell culture and transport studies

Caco-2 cells were grown in Dulbecco's Modified Eagle's medium containing 10% foetal calf serum and 1% non-essential amino acids, 2 mM L-glutamine, 100 units mL^{-1} penicillin G and 100 μg mL^{-1} streptomycin, as described previously (Tsuji et al 1994). For the transport experiments, Caco-2 cells were grown on the microporous polycarbonate membrane, Transwell (Costar, Bedford, MA, USA), and cultured for about 3 weeks before use in the transport experiments. Human myelogenous leukaemia K562 cells and their adriamycin-resistant variant K562/ADM cells, kindly supplied by Dr Tsuruo (Tokyo University, Tokyo, Japan), were cultured in RPMI 1640 medium containing 5% foetal calf serum, 100 μg mL^{-1} kanamycin and 2 mg mL^{-1} sodium bicarbonate (Tsuruo et al 1986). The adriamycin-resistant cells were maintained in culture medium containing 30 ng mL^{-1} adriamycin and were grown in drug-free medium 1 week before the experiments.

The transport study using Caco-2 cells grown on Transwell was performed as previously described (Tsuji et al 1994). Briefly, the confluent cells were washed with Hanks balanced salt solutions (HBSS) and 0·5 mL and 1·5 mL of HBSS were added on the apical and basolateral sides, respectively, of a cell insert. To measure apical to basolateral or basolateral to apical flux, a test compound was included in the apical or basolateral side, respectively. At the designated time, 0·5-mL samples of basolateral or 0·2-mL samples of apical side solution were sampled and subsequently equal volumes of HBSS were replaced. Uptake by K562 and K562/ADM cells was measured in transport buffer containing (mM): $CaCl_2$ 1·3, KCl 5·4, KH_2PO_4 0·44, $MgSO_4$ 0·81, NaCl 137, Na_2HPO_4 0·35, D-glucose 30 and HEPES 20 (pH 7·4). After 10-min preincubation of 1·5 × 10^6 cells in 0·25 mL transport buffer, 0·25 mL transport buffer containing test compound was added to initiate uptake experiments. At the designated time, 200-μL volumes of cell suspension were removed and placed in 400-μL microcentrifuge tubes containing a 100-μL layer of a mixture (density 1·022) of liquid paraffin (density 0·855) and silicone oil (density 1·068) in the ratio 1 to 3, and 50 μL of 3 M KOH (for radioactive test compound) or 3 M KCl (for non-radioactive test compound), and uptake was terminated by immediate centrifugation at 15 000 rev min^{-1}. After separation of the cells from the test solution by centrifugation, the cell pellet was used for the subsequent assay. For non-radioactive compounds, 150-μL volumes of incubation medium were added to the pellet fraction containing KCl after separation of cells from the medium. Cells precipitated were disrupted by ultrasonication and were filtered for HPLC analysis. For radioactive compounds, after separation of the cells from the medium, the pellet fraction containing KOH was neutralized with CH_3COOH (3 M; 50 μL), and radioactivity in the cell precipitate was then analysed as described below.

Measurement of intestinal absorption by the intestinal loop method

Male Wistar rats (Japan SLC, Hamamatsu, Japan) fasted overnight were anaesthetized by intraperitoneal injection of sodium pentobarbital (50 mg kg^{-1}), a midline incision was made to expose the small intestine. A 7·5-cm loop of jejunum approximately 3 cm distal to the ligament of Treitz was prepared by ligating both ends. The test compound, dissolved in isotonic phosphate buffer (pH 6·01, 0·5 mL), was injected into the jejunal loop, and the loop was maintained in the body for 1 h during the absorption experiment. At the end of the experiment, the test compound remaining in the intestinal loop was collected, the intestine was thoroughly washed with 20 mL isotonic phosphate buffer, and the amount of test compound recovered was quantitatively determined. Administration of cyclosporin was performed by three bolus intravenous injections of 6·25, 3·125 and 3·125 mg kg^{-1} doses at 0·30 and 60 sec, respectively, and subsequently intravenously infused at a constant rate (0·125 mg min^{-1} kg^{-1}) to maintain the plasma concentration. The unbound concentration of cyclosporin in plasma at these doses should be more than 0·4 μM when estimated from pharmacokinetic parameters and plasma-binding parameters (Bernareggi & Rowland 1991), which is higher than the P-glycoprotein inhibition constant of cyclosporin in intestinal epithelial cells, as described below.

Assessment of intestinal absorption during intestinal ischaemia

The rat intestine was exposed as described above. The superior mesenteric artery was isolated and a 30-cm loop of jejunum was exteriorized. The loop was attached to inflow and outflow tubes to enable perfusion with an isotonic phosphate buffer including a test compound at a flow rate of 0·2 mL min^{-1}. Outflow samples were collected every 30 min throughout the experiment. The intestinal segment and abdominal contents were kept moist with saline-soaked gauze to minimize tissue dehydration. The preparation was maintained at 37–38°C with a heat lamp. After the first 30 min intestinal perfusion, during which control permeability was evaluated, the superior mesenteric artery was occluded by ligation and the intestinal permeability of drugs was measured under ischaemic conditions for 120 min, until the end of experiment.

Analytical methods

To assay radio-labelled compounds, all samples were transferred into counting vials, mixed with scintillation fluid (Cleasol I; Nacalai Tesque, Kyoto, Japan) and measured with a liquid scintillation counter (Aloka, Tokyo, Japan). Non-radioactive compounds were measured by HPLC. The HPLC system was equipped with a constant flow pump (880-PU; Japan Spectroscopic, Tokyo, Japan), UV detector (875-UV; Japan Spectroscopic), fluorescence detector (RF-550; Shimazu, Kyoto, Japan), integrator (Chromatopac CR3A; Shimazu,

Kyoto, Japan) and automatic sample injector (AS-L350; Japan Spectroscopic). The analytical columns were reversed-phase TSK-gel ODS-80Ts (4·6 mm × 15 cm, Tosoh, Tokyo, Japan) for atenolol, nadolol, doxorubicin, sulphathiazole, sulphamethoxazole, digoxin, β-oestradiol and verapamil, and Wakosil-II 5C18AR (Wako Pure Chemicals, Osaka, Japan) for celiprolol, acebutolol and timolol. The mobile phases were 10 mM phosphate buffer containing 10 mM octanesulphonic acid-acetonitrile (65 : 35), 10 mM phosphate buffer containing 10 mM octanesulphonic acid methand-acetonitrile (30 : 20 : 50), 10 mM phosphate buffer-acetonitrile (50 : 50) water-acetonitrile (40 : 60), water-acetonitrile (90 : 10), 10 mM aqueous trifluoroacetic acid solution-methanol (30 : 70), 10 mM phosphate buffer containing 10 mM octanesulphonic acid-acetonitrile (70 : 30) and 10 mM acetate buffer-2-aminoheptane-acetonitrile (45·5 : 0·5 : 55·0) for celiprolol, digoxin, doxorubicin, β-oestradiol, sulphathiazole, sulphamethoxazole, timolol and verapamil respectively. The mobile phase for acebutolol, atenolol and nadolol was 10 mM phosphate buffer containing 10 mM octanesulphonic acid-methanol (50 : 50). The eluent was monitored at 235, 226, 323, 210, 225, 220, 268, 257, 294 and 270 nm for acebutolol, atenolol, celiprolol, digoxin, β-oestradiol, nadolol, sulphamethoxazole, sulphathiazole, timolol and verapamil, respectively. Doxorubicin was detected with a fluorescence detector at $\lambda_{ex.} = 480$ nm and $\lambda_{em.} = 530$ nm. ATP was measured by the luciferine-luciferase reaction using an ATP assay kit (Wako Pure Chemicals, Osaka, Japan). Cellular protein was measured by the method of Lowry et al (1951) with bovine serum albumin as the standard.

Data analysis

The uptake (μL/1·5 × 10^6 cells) and permeability (μL (mg protein)$^{-1}$) of compounds were calculated by dividing the amount transported (dpm or area/1·5 × 10^6 cells or mg protein) by the initial concentration of test compounds on the donor side (dpm or area per μL). The permeability coefficient (μL min^{-1} (mg protein)$^{-1}$) was obtained by linear regression analysis of the slope of the initial linear portion of the plots of permeability against time (min). Uptake amount or amount permeated can be obtained by multiplying by the concentration of the compounds.

In the experiment on drug absorption by intestinal loops the amount absorbed was determined by subtracting the amount of the test compound remaining in the isolated loops from the total amount injected. The first-order absorption rate constant, k_a was calculated as follows:

$$A_t = A_0 e^{-ka \cdot t}$$

where A_0 and A_t are, respectively, the amount of test compound administered, and the amount recovered from the loops at time t.

The apparent absorption clearance ($CL_{abs,app}$) of test compounds assessed by intestinal single pass perfusion was calculated as follows:

$$CL_{abs,app} = Q \times (C_{in} - C_{out})/C_{in}$$

where Q is the flow rate of the perfusate (mL min^{-1}), and C_{in} and C_{out} are the concentrations of the test compounds in the inflow and outflow perfusate, respectively. The true absorption clearance (CL_{abs}) was obtained after correcting $CL_{abs,app}$ for the absorption clearance of [^{3}H]PEG900 obtained in the same manner as $CL_{abs,app}$.

All data are expressed as means ± s.e.m., and statistical analysis was performed by using Student's one-tailed *t*-test. The level of significance was taken to be $P < 0.05$.

Results

Uptake of β-blockers by drug-sensitive and drug-resistant cancer cells

In order to learn whether several drugs, including β-blockers such as acebutolol, celiprolol, nadolol and timolol, can be substrates of P-glycoprotein, their accumulation by human leukaemic drug-sensitive K562 cells and their multidrug-resistant variant K562/ADM cells was examined. The uptake of β-blockers was measured at steady-state, 60 min, and is shown in Table 1. The uptake of all β-blockers by drug-resistant K562/ADM cells was significantly less than by sensitive K562 cells.

The effect of multidrug-resistance reversing agents on acebutolol uptake by K562 and K562/ADM cells was also examined. The results are shown in Table 2. When acebutolol uptake was measured in the presence of monoclonal antibody against P-glycoprotein, MRK16, uptake by K562/ADM cells was significantly increased, whereas uptake by K562 cells was unchanged. Specific multidrug-resistance reversing agents MS-209 and cyclosporin also increased the uptake of acebutolol by K562/ADM cells in a concentration-dependent manner, with even greater accumulation of acebutolol at higher concentrations (10 μM) than lower concentrations (1 and 0·5 μM for MS-209 and cyclosporin, respectively). Although increases were also observed for acebutolol uptake by drug-sensitive K562 cells, the extent of the increase was much smaller than observed in drug-resistant K562/ADM cells. A similar effect of cyclosporin was also observed on the steady-state uptake of quinidine by K562/ADM cells.

Transport across Caco-2 cells

Vectorial transport of various compounds across a monolayer of Caco-2 cells was examined; the results are shown in Fig. 1. Apical to basolateral ($J_{ap \rightarrow bl}$) and basolateral to apical ($J_{bl \rightarrow ap}$) transport rates of acetamide, which is transported through Caco-2 monolayers transcellularly by passive diffusion, were comparable. The values were $J_{ap \rightarrow bl} = 1{\cdot}22 \pm 0{\cdot}04$ and $J_{bl \rightarrow ap} = 1{\cdot}36 \pm 0{\cdot}07$ μL min^{-1} (mg protein)$^{-1}$. Similarly, no polarized flux was observed for paracellular transport evaluated by [^{3}H]mannitol transport, with values of $J_{ap \rightarrow bl} = 0{\cdot}16 \pm 0{\cdot}04$ and $J_{bl \rightarrow ap} = 0{\cdot}18 \pm 0{\cdot}05$. In contrast, the $J_{bl \rightarrow ap}$ values for [^{3}H]vinblastine and acebutolol

Table 1. Uptake of β-blockers by K562 and K562/ADM cells.

Compound	Concn (μM)	Uptake (μL/1·5 × 10^6 cells) K562	K562/ADM
Acebutolol	500	2·7 ± 0·4	1·0 ± 0·1*
Celiprolol	500	2·0 ± 0·1	0·8 ± 0·1*
Nadolol	500	1·8 ± 0·1	0·9 ± 0·1*
Timolol	100	6·5 ± 1·1	4·1 ± 0·1*

Mean ± s.e.m. of 3 or 4 experiments. *$P < 0.05$ compared with

Table 2. Effect of various MDR reversing agents on the uptake of acebutolol and quinidine by K562 and K562/ADM cells.

	Uptake (μL/1·5 × 10^6 cells)			
	K562		K562/ADM	
Acebutolol				
+mouse IgG (control)	2·02 ± 0·33	(1)	0·28 ± 0·02*	(1)
+MRK 16	2·08 ± 0·17	(1·03)	0·58 ± 0·11*	(2·1)
+MS-209 (control)	2·25 ± 0·14	(1)	0·20 ± 0·07*	(1)
+ MS-209, 1 μM	2·49 ± 0·18	(1·11)	0·73 ± 0·08*'**	(3·7)
+MS-209, 10 μM	2·95 ± 0·36	(1·31)	1·21 ± 0·14*'**	(6·05)
+cyclosporin (control)	1·99 ± 0·11	(1)	0·18 ± 0·04*	(1)
+cyclosporin, 0·5 μM	2·37 ± 0·49	(1·19)	0·49 ± 0·14*	(2·7)
+cyclosporin, 10 μM	2·68 ± 0·09	(1.35)	2·03 ± 0·48**	(11·3)
[^{3}H]Quinidine				
none (control)	31·10 ± 2·13	(1)	4·66 ± 0·20*	(1)
+cyclosporin, 10 μM	24·81 ± 0·71**	(0·80)	21·74 ± 0·58**	(4·67)

Uptake of acebutolol (500 μM) and [^{3}H]quinidine (6·6 nM) was measured in the presence and absence of various MDR reversing agents at 37°C, as described in Materials and Methods. Each value represents the mean ± s.e.m. of 3 or 4 experiments. The values shown in parentheses represent the magnitude of the increase compared with each of the controls. *$P < 0·05$; significant difference between uptake by K562 and K562/ADM cells. **$P < 0·05$; significantly different from the respective control value.

(0·313 ± 0·02 and 0·048 ± 0·003, respectively) were significantly higher than $J_{ap \rightarrow bl}$ values for [^{3}H]vinblastine and acebutolol (0·052 ± 0·006 and 0·025 ± 0·001, respectively).

Table 3 shows the effect of metabolic inhibitors on the transport of acebutolol across Caco-2 cells. Caco-2 cells were treated with 10 mM sodium azide and NaF for 20 min to deplete ATP in the cells. Treatment of the cells with two metabolic inhibitors induced a significant decrease in the $J_{bl \rightarrow ap}$ value to approximately half, without any effect on apical to basolateral transport.

Effect of cyclosporin on intestinal absorption

Intestinal absorption of acetamide, acebutolol and vinblastine in rats was evaluated by the in-situ intestinal loop method. Each drug was administered into the jejunum at a concentration of 50 μM. The effect of cyclosporin was examined by bolus and constant intravenous administration. Intestinal absorption expressed as percentages of the doses of acebutolol and vinblastine was increased significantly by intravenous administration of cyclosporin, from 15·9 ± 2·5 to 36·3 ± 5·7 and from 9·9 ± 1·7 to 22·4 ± 5·4, respectively, whereas

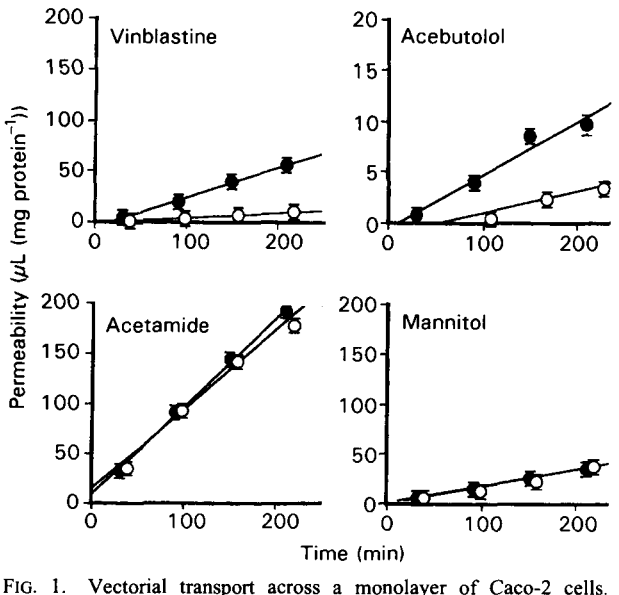

FIG. 1. Vectorial transport across a monolayer of Caco-2 cells. Permeability of [^{3}H]vinblastine (50 nM), acebutolol (100 μM), [^{14}C]acetamide (14 μM) and [^{3}H]mannitol (30 μM) from the apical to the basolateral side (○) and from the basolateral to the apical side (●) was measured at 37°C. Each point represents the mean ± s.e.m. of three experiments.

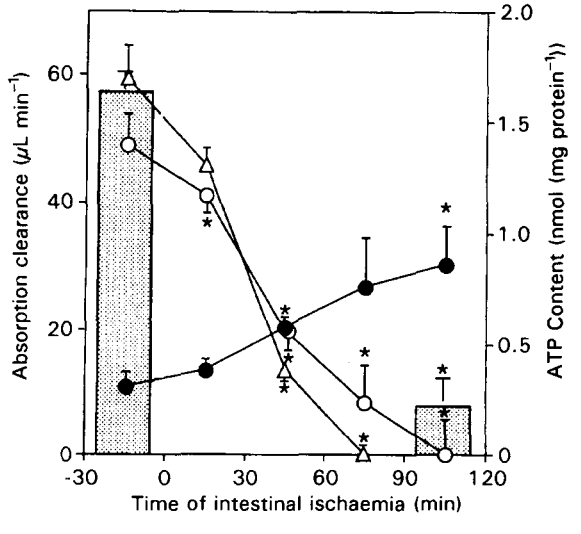

FIG. 2. Effect of ischaemia on intestinal absorption and tissue ATP content. Absorption clearance of [^{3}H]vinblastine (600 pM, ●), [^{3}H]3-*O*-methylglucose (80 pM, ○) and [^{14}C]acetamide (36 nM, △) was measured under normal and ischaemic conditions and is plotted at the midpoint of the sample collection intervals. The tissue ATP-content of the jejunum was estimated before and 120 min after ischaemia. Each symbol represents the mean ± s.e.m. of 3–6 experiments. *$P < 0·05$; significantly different when compared with normal conditions.

Table 3. Effect of metabolic inhibitors on transport of acebutolol across Caco-2 cell monolayers.

	Permeability coefficient (μL min^{-1} (mg protein)$^{-1}$)	
	Apical to basolateral	Basolateral to apical
Control	0·008 ± 0·001	0·030 ± 0·004
+ 10 mM NaN_3, + 10 mM NaF	0·011 ± 0·003	0·015 ± 0·006*

Caco-2 cells were preincubated in the presence or absence (control) of 10 mM NaN_3 and 10 mM NaF for 20 min. Transport of acebutolol (100 μM) was measured at 37°C for 210 min in the presence or absence (control) of metabolic inhibitors. Each value represents the mean ± s.e.m. of 3 experiments. *$P < 0.05$; significantly different from the control value.

absorption of acetamide remained unchanged (from 71·9 ± 6·7 to 70·5 ± 2·2).

Effect of intestinal ischaemia on intestinal absorption

The effect of intestinal ischaemia, induced by occluding the superior mesenteric artery, on intestinal absorption of 3-*O*-methylglucose, acetamide and vinblastine was examined. Intestinal absorption was evaluated by jejunal single pass perfusion of each compound. The ATP content of intestinal tissue was measured to quantify the intestinal ischaemic state. Occlusion of the artery for 120 min led to a significant reduction in ATP content, from 1·58 ± 0·11 nmol (mg protein)$^{-1}$ to 0·23 ± 0·10 nmol (mg protein)$^{-1}$. As shown in Fig. 2, absorption of [^{3}H]3-*O*-methylglucose decreased in a time-dependent manner during ischaemia. A statistically significant decrease in intestinal absorption clearance, from 48·9 ± 5·5 μL min^{-1} to 19·8 ± 3·5 μL min^{-1}, was observed within 60 min of the start of ischaemia. After 120 min ischaemia, intestinal absorption clearance of [^{3}H]3-*O*-methylglucose was completely abolished, diminishing to 0·1 ± 5·6 μL min^{-1}. Intestinal absorption clearance of [^{14}C]acetamide was also reduced during the ischaemic state. In contrast, absorption clearance of [^{3}H]vinblastine was increased by occlusion of the artery in a control study (10·8 ± 2·3 μL min^{-1}) to 20·4 ± 1·4 and 30·3 ± 5·2 μL min^{-1} at 60 and 120 min, respectively, after occlusion.

Discussion

In this study, it was demonstrated using four different experimental techniques, i.e. cellular uptake by drug-sensitive and multidrug-resistant cancer cells, transcellular transport through human intestinal cell line Caco-2, the in-vivo intestinal loop method and the intestinal ischaemic rat model, that the previously observed lower oral bioavailability and secretion from blood to intestinal lumen of β-blockers is partially ascribable to the contribution of P-glycoprotein.

The difference between steady-state uptake of β-blockers in multidrug-resistant cancer cells, K562/ADM cells, which express an excess of P-glycoprotein (Tsuruo et al 1986), and drug-sensitive K562 cells, suggests that the β-blockers acebutolol, celiprolol, nadolol and timolol, are transported out of cells by P-glycoprotein (Table 1). That these β-blockers have affinity for P-glycoprotein is confirmed by the effects of specific P-glycoprotein inhibitors. Monoclonal antibody MRK16 is known to inhibit the activity of P-glycoprotein (Naito et al 1993). Although an effect of MRK16 on the increment of acebutolol uptake was observed in the drug-resistant cells, no significant effect of the antibody was observed in the drug-sensitive cells. Accordingly, the effect of MRK16 is attributed to specific inhibition of P-glycoprotein-mediated efflux. Cyclosporin and MS-209 also specifically bind to P-glycoprotein, thereby reducing drug transport mediated by this compound (Tamai & Safa 1990; Sato et al 1995). Although they increased the uptake of acebutolol by both drug-sensitive and drug-resistant cells, their effect on drug-resistant cells was much greater than on drug-sensitive cells. These observations demonstrate clearly for the first time that the β-blockers tested in this study are transported by P-glycoprotein. The greatest decrement in steady-state uptake by drug-resistant cells compared with drug-sensitive cells was observed with acebutolol. This might mean that acebutolol has the highest affinity for P-glycoprotein among these β-blockers.

Unidirectional transport of vinblastine from the basolateral to the apical side (Fig. 1) is consistent with previous reports showing a functional contribution of P-glycoprotein in Caco-2 cells (Karlsson et al 1993). Such unidirectional transport in Caco-2 cells is supposed to be caused by specific mechanisms, because the transport of acetamide (passive diffusion) and mannitol (paracellular transport) were not polarized. Similarly, acebutolol exhibited unidirectional flux, and only basolateral to apical flux was reduced by treating Caco-2 cells with metabolic inhibitors (Table 3). These observations in Caco-2 cells are consistent with the functional characteristics of the P-glycoprotein present at the apical membrane in eliminating drugs from cells.

There have been several studies demonstrating drug transport mediated by P-glycoprotein in intestinal tissue (Meyers et al 1991; Hsing et al 1992). They include measurement of polarized transport from the serosal to the mucosal side across intestinal tissue and the effect of specific P-glycoprotein inhibitors such as verapamil on transepithelial transport (Hsing et al 1992). In the present study, the functional contribution of P-glycoprotein-mediated transport in-vivo was evaluated in two different experiments in terms of specific inhibition by cyclosporin and the ATP-dependence of P-glycoprotein-mediated transport.

Inhibition by cyclosporin was assessed by simultaneous intravenous administration of cyclosporin while measuring intestinal absorption by the in-situ jejunal loop method. Cyclosporin is an effective inhibitor of P-glycoprotein with a relatively low inhibitory constant of about 0·08 μM (Naito & T

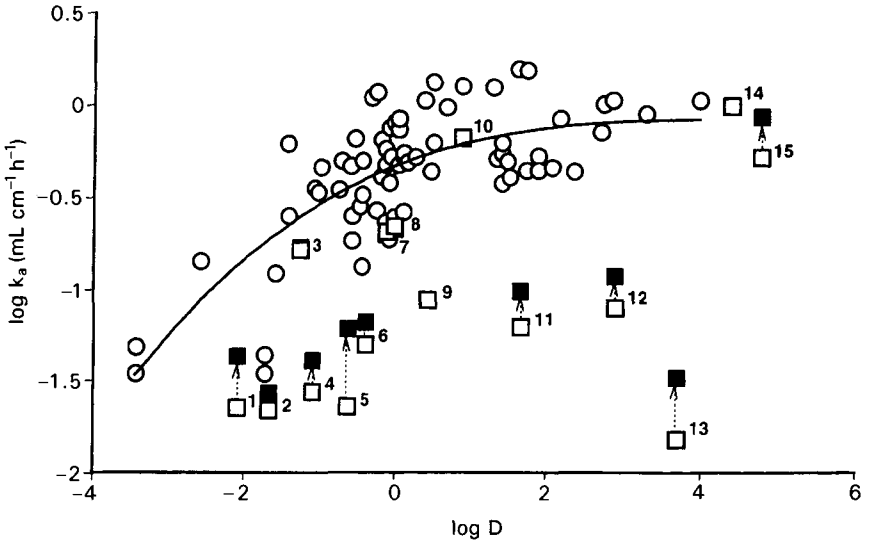

FIG. 3. Summary of the relationship between first-order rate constants for the intestinal absorption and lipid solubility of various drugs. The results shown in the squares represent the relationship between intestinal absorption rate constants (k_a) obtained by the in-situ jejunum loop method with (■) and without (□) cyclosporin in rats and octanol-buffer (pH 7·0) partition coefficients (log D) determined in this study. The results shown in circles were reported by Komiya et al (1980), Martin et al (1981) and Taylor et al (1985). 1, atenolol; 2, nadolol; 3, [^{14}C]acetamide; 4, celiprolol; 5, acebutolol; 6, doxorubicin; 7, timolol; 8, sulphathiazole; 9, [^{3}H]quinidine; 10, sulphamethoxazole; 11, digoxin; 12, [^{3}H]cyclosporin; 13, [^{3}H]vinblastine; 14, β-oestradiol; 15, verapamil.

suruo 1989). From the pharmacokinetic parameters reported (Bernareggi & Rowland 1991) and the plasma-unbound fraction of cyclosporin at steady-state in rats, the expected intestinal epithelial-free concentration of cyclosporin at steady-state is more than 0·4 μM according to the dosage schedule used in this study. Accordingly, the function of P-glycoprotein-mediated transport of the test compounds should be significantly disrupted by cyclosporin. Intestinal absorption of acebutolol and vinblastine increased 2·6 and 2·2 times, respectively, in response to administration of cyclosporin, whereas acetamide absorption was unaffected. Accordingly, it can be said that P-glycoprotein contributes significantly to the reduction of intestinal epithelial permeability.

The ATP-dependence of P-glycoprotein-mediated transport was determined by manipulating the ATP content of intestinal tissue by intestinal ischaemia. A rat intestinal ischaemic model was prepared by occluding the superior mesenteric artery and intestinal absorption was subsequently evaluated by single-pass perfusion of test compounds. The ischaemic state was evaluated by monitoring the ATP content of intestinal tissue. By using a similar ischaemic model in rat brain, we successfully demonstrated a P-glycoprotein related to the restricted distribution of cyclosporin and doxorubicin into the brain (Sakata et al 1994; Ohnishi et al 1995). In the present intestinal ischaemic model, ATP content was markedly reduced by occlusion of the mesenteric artery for 120 min. As ATP content diminished, intestinal absorption of 3-*O*-methylglucose, which crosses the intestinal brush-border membrane by a sodium-dependent secondary active transport mechanism (Hediger et al 1987), decreased. Absorption clearance of passively transported acetamide was also reduced by ischaemia; this is presumably ascribable to accumulation of acetamide in the vasculature by the lack of blood flow, resulting in a reduced concentration gradient. In contrast, vinblastine, for which unidirectional transport from the basolateral (serosal) to the apical (mucosal) side has been reported in Caco-2 cells (Hunter et al 1993; Wils et al 1994) and in the present study (Fig. 1), showed increased intestinal absorption in ischaemia in a time-dependent manner (Fig. 2). Accordingly, the increase in vinblastine transport is specifically affected by the reduction in ATP content, and P-glycoprotein contributes to the lower bioavailability of vinblastine, and also presumably of other drugs which have affinity for P-glycoprotein, including β-blockers.

These results clearly show that P-glycoprotein functions in the rat small intestine and suggest that it reduces apparent intestinal epithelial penetration of many of drugs from the lumen to the blood. Fig. 3 summarizes the relationship between first-order rate constants of intestinal absorption evaluated by intestinal perfusion or loop methods and the lipid-solubility of the drugs. The data include the β-blockers, atenolol, acebutolol, celiprolol and nadolol, the anticancer drugs, doxorubicin and vinblastine and other types of drug, cyclosporin, digoxin and verapamil. The dotted line represents the eye-fitted correlation curve for the drugs shown in the circles. The intestinal absorption of the drugs involved in the multi-drug-resistance phenotype, doxorubicin, vinblastine and verapamil, as well as cyclosporin and some β-blockers tend to be increased to some extent by cyclosporin administration, although the increased rate constants still seem lower than the obtained correlation (dotted line).

In conclusion, P-glycoprotein reduces intestinal permeability to some drugs, although the contribution of other mechanisms limiting their intestinal permeability cannot be ruled out.

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