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Relevance of p-glycoprotein for the enteral absorption of cyclosporin A: *in vitro*–*in vivo* correlation

[1]G. Fricker, *J. Drewe, #J. Huwyler, *H. Gutmann & *C. Beglinger

Institute of Pharmaceutics and Biopharmacy, D-69120 Heidelberg, Germany; *Medical Outpatient Clinic and Division of Gastroenterology, Kantonsspital, CH-4031 Basel and #Department of Anaesthesia and Research, University Hospital, CH-4031 Basel, Switzerland

1 The interaction of cyclosporin A (CyA) with p-glycoprotein during intestinal uptake was investigated by a combination of *in vitro* experiments with human Caco-2 cells and an intubation study in healthy volunteers.

2 CyA uptake into the cells was not saturable and exhibited only a low temperature sensitivity, suggesting passive diffusion. When the permeation of CyA across Caco-2 monolayers from the apical to the basolateral side was determined, overall transport had an apparently saturable component up to a concentration of 1 μM. At higher concentrations permeation increased over-proportionally. Calculation of the kinetic parameters of apical to basolateral permeation suggested a diffusional process with a K_D of 0.5 μl min^{-1} per filter, which was overlayed by an active system in basolateral to apical direction with a K_M of 3.8 μM and a J_{max} of 6.5 picomol min^{-1} per filter.

3 CyA permeation was significantly higher when the drug was given from the basolateral side as compared to the permeation from the apical side. Apical to basolateral transport of CyA was increased in the presence of vinblastine, daunomcyin and a non-immunosuppressive CyA-derivative. All compounds inhibit p-glycoprotein-mediated transport processes. Basolateral to apical permeation of CyA showed a dose-dependent decrease in the presence of vinblastine. Permeation of daunomycin across Caco-2 cell monolayers was also higher from the basolateral to the apical side than *vice versa*. Basolateral to apical permeation was decreased in the presence of SDZ PSC 833 and cyclosporin A.

4 Western blot analysis of Caco-2 cells with the monoclonal antibody C219 confirmed the presence of p-glycoprotein in the used cell system.

5 When the absorption of CyA in the gastrointestinal (GI)-tract of healthy volunteers was determined, a remarkable decrease of the plasma AUC could be observed dependent on the location of absorption in the rank order stomach > jejunum/ileum > colon. The decrease in absorption exhibited a marked correlation ($r = 0.994$) to the expression of mRNA for p-glycoprotein over the GI-tract (stomach < jejunum < colon).

6 All data provide evidence that CyA is a substrate of p-glycoprotein in the GI-tract, which might explain the local differences and the high variability in cyclosporin absorption found *in vivo*.

Keywords: Cyclosporin A; p-glycoprotein; Caco-2 cells; intestinal absorption; intubation study; local administration

Introduction

The endecapeptide cyclosporin A (CyA) is of particular clinical interest because of its immunosuppressive properties allowing an effective treatment of autoimmune diseases and prevention of allograft rejection after organ transplantation (Borel *et al.*, 1976; Cohen *et al.*, 1984; Rogers & Kahan, 1984; Kahan, 1989). The preferred way of administration during long term treatment is via the oral route. However, without using optimized drug formulations, the absorption of CyA in man appears to be quite variable. Absolute bioavailabilities between <5% and 89% have been observed in patients (Ptachinski *et al.*, 1985). In rats, bioavailabilities between 10% and 30% have been determined after administration of the drug (Ueda *et al.*, 1984; Wassef *et al.*, 1985; Lindberg-Freijs & Karlsson, 1994). Thereby, the rate and extent of cyclosporin A absorption increased with increasing doses. This observation was attributed to effects of the olive oil-formulation upon gastric emptying and/or metabolic events in the gastrointestinal tract. Other studies, however, have demonstrated a high stability of CyA in the gastrointestinal tract (Calne *et al.*, 1978).

It might well be that other determinants regulating the variability and nonlinearity of CyA pharmacokinetics include physiological factors, such as specific transport systems in epithelial cells. Along this line of investigation we have previously shown that secretion of cyclosporins in intact proximal kidney tubules is modulated by p-glycoprotein, a member of the ATP-dependent multidrug export pump family (Schramm *et al.*, 1995). Our findings were supported by studies with a transfected porcine kidney cell line, LLC-GA5-COL300, which overexpresses p-glycoprotein, demonstrating an increased transport of CyA as compared to the normal LLC-PK$_1$ cell line (Saeki *et al.*, 1993). Other studies have provided additional evidence for a p-glycoprotein-mediated transport of CyA at the blood-brain barrier and inhibition of p-glycoprotein function by CyA in the secretory membrane of hepatocyte couplets (Takeguchi *et al.*, 1993; Sakata *et al.*, 1994). Further evidence has derived from experiments that show inhibition of p-glycoprotein-mediated transport of vinblastine across monolayers of an intestinal carcinoma cell line (HCT-8) by CyA and its non-immunosuppressive analogue SDZ PSC 833 (Zacherl *et al.*, 1994). Based on these observations of interaction we intended to investigate whether p-glycoprotein also modulates the intestinal absorption of cyclosporins. First, we measured the permeation of the drug in an *in vitro* cell culture model (Caco-2 cell monolayers) to assess specific transport systems and second we quantified drug absorption in the human gastrointestinal (GI)-tract after intubation and perfusion of the drug to various parts of the gut.

[1]Author for correspondence at: Institute for Pharmaceutics and Biopharmacy, Im Neuenheiuer Feld 366, D-69120 Heidelberg, Germany.

Methods

Cell culture

Caco-2 cells (passages 70–90) were grown on Costar Snapwell polycarbonate filters, having a pore size of 0.45 μm. Supplemented Dulbecco's modified Eagle's medium containing 10% (v/v) foetal calf serum was used as culture medium and as transport medium. After 14–17 days of culture, confluency of the cell monolayers was achieved as determined by measurement of the transepithelial resistance (350–400 Ω cm^2) and permeability of the extracellular marker PEG-4000 (0.1–0.25% of a given dose). Functional integrity of the cells was tested by measuring directed taurocholate and phenylalanine permeation across the monolayers. Both compounds exhibited saturable and temperature-dependent permeation from the apical to the basolateral compartment, which is consistent with data described in the literature (Hidalgo & Borchardt, 1990a,b).

Sublines of murine monocytic leukaemia P388 cells, one parental (Par-P388) and one multidrug-resistant (MDR-P388), which were used as reference cell lines for the determination of p-glycoprotein, were cultured as described previously (Boesch *et al.*, 1991).

Enzyme and protein determinations

Alkaline phosphatase activity was determined as described previously (Keeffe *et al.*, 1979) with p-nitrophenylphosphate as substrate. Protein was determined by a colour reaction (Bradford, 1976) with bovine γ-globulin as standard.

Immunodetection of P-glycoprotein

P-glycoprotein was detected by Western blot analysis with the monoclonal antibody C219 (Centocor, Malvern, PA, U.S.A.). SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with a Mini-Protean II apparatus (Bio-Rad, Hercules, CA, U.S.A.). To epitheliall cell homogenates (1 mg ml^{-1} protein) 1/5 volume sample buffer (10% glycerol, 5% SDS, 40 mM DTT, 0.00625% bromophenol blue, 62.5 mM Tris/HCL pH 6.8) was added. The samples were agitated 30 min at 25°C and loaded onto 6.5% acrylamide/bisacrylamide gels. After electrophoresis, proteins were transferred electrophoretically (2 h at a constant amperage of 2 mA/cm^2) to a 0.45 μm pore size nitrocellulose membrane by use of a Mini Trans-Blot cell (Bio-Rad). The transfer buffer contained 192 mM glycine, 25 mM Tris and 20% methanol. The membrane was blocked overnight at 4°C with 5% powdered skimmed milk in Tris-buffered saline (50 mM Tris and 150 mM NaCl) containing 0.3% Tween 20 (TBS-T). Washed membranes were incubated with Mab C219 (200 ng ml^{-1}) in TBS-T, 1% bovine serum albumin (BSA) and 0.05% NaN_3 for 2 h at 37°C. Washed membranes were incubated for 1 h at room temperature with horseradish peroxidase-conjugated rabbit anti-mouse IgG (1:1000) (Dako, Glostrup, Denmark) in TBS-T containing 2% milk powder. Membranes were washed in TBS-T and P-gp was visualized by enhanced chemiluminescence detection (ECL-kit by Amersham, Buckinghamshire). Molecular weights were determined in comparison with Bio-Rad standards: myosin (206 kDa), β-galactosidase (117 kDa), bovine serum albumin (895 kDa) and ovalbumin (47 kDa).

Transport experiments

The cell monolayers grown on polycarbonate filters were fixed in side-by-side diffusion chambers (Snapwell System, Costar) and kept at 37°C. For uptake studies the cultured cells were incubated with the labelled drug at the concentrations indicated in the figure legends. After certain time intervals the monolayer-covered filters were removed from the diffusion chambers, the cells were washed twice with ice-cold incubation medium without measured substrate and the cell associated radioactivity was determined by liquid scintillation counting. The diffusion chamber allowed a constant mixing of the media on both sides by an air-lift system. The pH on both sides was kept constant by gassing apical and basolateral compartment via the air-lift with carbogen (5% CO_2, 95% O_2). In permeation experiments the cell monolayers were incubated from either the apical or the basolateral side with the respective labelled compound (concentrations see figure legends) in a volume of 4 ml in the absence or in the presence of competitors. The compounds were given dissolved in DMSO or ethanol not exceeding a volume of 50 μl. All reference incubations were done with the same amounts of pure solvent. At 15 min time intervals aliquots of 200 μl were taken from the opposite chamber compartment and replaced by 200 μl incubation medium. The amount of permeated drug in the samples was determined by liquid scintillation counting after correction for changes of volume and concentrations by replacement of media.

The kinetic parameters of CyA permeation across the monolayers were analyzed by the nonlinear least-squares regression analysis program ENZFITTER 1.05 (Elsevier-BIOSOFT, Cambridge) in the J-against-C diagram taking all data points into account with the same weight. The resulting data are presented as means ± s.e.mean.

Intubation study with healthy volunteers

The study was performed with 10 male volunteers, ages between 20 and 45 years and body weights of 56–83 kg. The study was performed in accordance with the guidelines of the Declaration of Helsinki as revised in Tokyo (1975) and in Venice (1983). The study protocol and informed consent forms were approved by the Human Ethics Committee of the Kantonsspital Basel. No clinically significant abnormalities could be detected by physical examination including measurements of pulse rate, blood pressure and laboratory investigations. Each subject obtained 150 mg CyA as a single oral dose in a randomized open-label, five period, Latin-square design. The drug was given in a solution containing polyethylene glycol castor oil, middle chain triglycerides and low molecular weight glycols. The wash-out period between the administrations was at least 7 days. CyA was administered to different parts of the GI-tract by gavage (stomach, jejunum/ileum, colon). Fourteen hours before the small intestinal administrations, the volunteers were intubated with a modified on-lumen GI tube (Cartmill feeding tube, Hollander Medizin-Technik, Cham, Switzerland), with an inner diameter of approximately 1.5 mm, an outer diameter of about 2.5 mm and a length of 3.5 m. The tube was placed under fluoroscopic control and was allowed to be transported distally by peristaltic gut movement. Retrograde insertion of tubes into the colon was performed 15 min before drug administration with minimal air insufflation but without cathartics or enemas. All subjects underwent rigid sigmoidoscopy for the placement of the GI-tube. The tube was placed in the left colon about 30 cm proximal to the anus. Drug solutions were injected into the tubes and then 15 ml (10 ml for the colon) of saline solution was injected into the tubes over 15 min. Before the study, the adsorption of CyA to the tubing was measured after injection of the drug formulation through the tube. Binding to the plastic material was less than 5% of the administered dose. All administrations were done after an overnight fast of 10 h with only water allowed. Food was not allowed for the first 4 hours after drug administration. Then, a standard liquid meal of a caloric content of 2090 kJ (500 ml Ensure, Abbott, Cham, Switzerland) was given. Blood samples were taken before and up to 32 h after drug administration. Whole blood samples were analyzed for CyA concentrations by use of the specific monoclonal Sandimmun radioimmunoassay (Ball *et al.*, 1988) with a detection limit of 15 ng ml^{-1}.

Chemicals

[^{3}H]-cyclosporin A with a specific activity of 9.3 Ci mmol^{-1}, and the cyclosporin D analogue (3'keto-Bmt^1)-[Val]^2-cyclo sporin, SDZ PSC 833, were kindly provided by Sandoz Pharma, Basle, Switzerland. [^{3}H]-phenylalanine with a specific activity of 50 Ci mmol^{-1} and [^{14}C]-polyethylene glycol 4000 with a specific activity of 14 mCi g^{-1} were from Amersham. The radiochemical purity of all labelled compounds was determined by high-performance thin-layer chromatography before the experiments and was found to be greater than 98%. Caco-2 cells, originally derived from a human colorectal carcinoma, were purchased from the American Type Culture Collection, Rockville, Maryland, U.S.A. All other chemicals were reagent grade and were obtained from commercial sources.

Statistical analysis

Maximum whole blood concentrations (C_{max}) were compiled from the raw data. Areas under the blood concentration-time curves (AUC) up to 32 h were calculated by use of the trapezoidal rule. Blood drug concentrations below the assay limit of 15 ng ml^{-1} were assumed to be zero. Sample differences were tested for normal distribution by the Wilk-Shapiro test and the homogeneity of variances by Levene's test (RS1 software package, 1988). At normal distribution, samples were tested by two-way analysis (GLM, SAS software package, 1988), otherwise, analysis was applied to rank-transformed data by the Friedman test (Conover & Iman, 1981). For significant differences analysis of variance was done by the Newman-Keuls test for pairwise comparison (SAS software package, 1988).

Results

The functional properties of Caco-2 cells, which served as an *in vitro* model for the intestinal barrier, were evaluated by determination of the permeability of the paracellular marker polyethylene glycol 4000 and the actively transported compounds taurocholic acid and phenylalanine. The amount of [^{14}C]-polyethylene glycol 4000 permeating the cell monolayers between days 14 and 17 varied between 0.1 and 0.25% per hour of an administered tracer dose pointing to confluency of the monolayer. The determination of alkaline phosphatase activity in the cells revealed an 8 fold increase of activity between days 4 and 14 of culture indicating the formation of polarized cells in the monolayer with a high expression of the enzyme in the apical membrane. This corresponds well to previous observations demonstrating an 11.4 fold increase of activity in the brush border membrane fraction of cells cultured between day 5 and 19 (Pinto *et al.*, 1983). The permeation of taurocholic acid from the apical to the basolateral side was higher than the permeation rate from the basolateral to the apical side. This indicates the formation of polar-organized cells, which exhibit active transport of bile salts predominantly from the apical to the basolateral side. Permeation of taurocholate from the apical to the basolateral side was saturable. For that saturable permeation an apparent K_M of 180 ± 45 μM and a V_{max} of 9.8 ± 1.4 picomol min^{-1} per incubation dish was calculated under the assumption that the saturable transport follows Michaelis-Menten-kinetics. These values are in accordance with previously observed data (Hidalgo & Borchardt, 1990b). Thus, the result corresponds to directed bile acid transport occurring in intestinal cells (Marcus *et al.*, 1991). Similar observations were made when phenylalanine was used as a substrate for permeation.

The uptake of CyA into the Caco-2 cell monolayers was determined at concentrations of 1, 2, 5 and 10 μM. In that concentration range no saturability of transport was observed. Higher concentrations were not used because of the poor solubility of CyA. When the incubation temperature of the cells was decreased from 37°C to 20°C only a slight decrease in transport rates was observed. In contrast, control experiments in which amino acid transport was measured demonstrated the phenylalanine transport to be saturable and temperature-sensitive, indicating the functional integrity of active uptake processes in our test system. The lack of saturability and the low temperature sensitivity of CyA transport suggest that only passive diffusion is involved in the cellular uptake of the CyA. When concentrations below 1 μM were used, a deviation from linearity was observed. However, the variability of the measured values did not allow a precise determination of the potential involvement of an active transport system. Therefore, overall permeation of CyA through the cell monolayer was determined.

When the permeation of [^{3}H]-CyA through the cell monolayer from the apical to the basolateral compartment was measured at concentrations of 0.1, 0.2, 0.5, 1, 2, 5 and 10 μM an apparent saturability of permeation could be seen up to a concentration of 1 μM. But, at higher concentrations the permeation rate was comparable to the findings described above. It was significantly higher than would be expected for the shape of the curve of a saturable transport, which was assumed from the measured values at the lower concentrations (Figure 1). Therefore, the measured values were analyzed under the assumption that CyA permeates the cells in the apical to basolateral direction by a diffusional transport, which is superimposed by an active transport system operating into the opposite direction. Non-linear regression analysis revealed a diffusion constant K_D of 0.5 μl min^{-1} per filter, which was overlayed by an active and saturable transport system in the basolateral to apical direction with a K_M of 3.8 μM and a J_{max} of 6.5 picomol min^{-1} per filter.

The cell monolayers were also incubated from the basolateral side with CyA in order to determine transport rates of the drug given from that side. Direct comparison of the transport rates at a given concentration showed that basolateral to apical permeation was higher than apical to basolateral permeation (Figure 2), suggesting the involvement of a

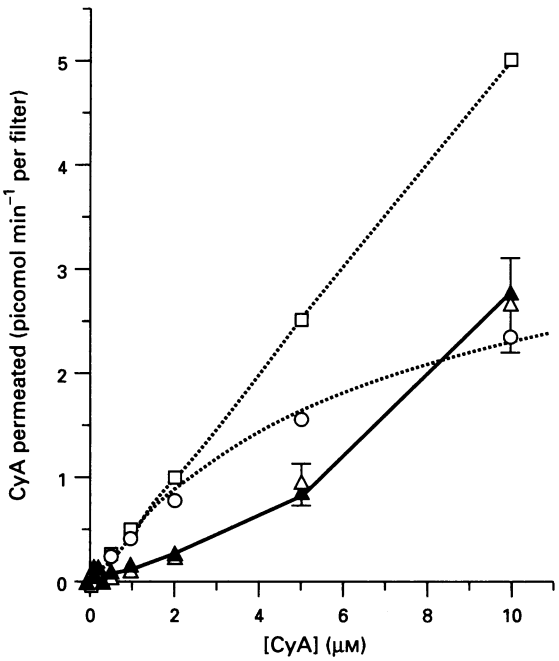

Figure 1 The dose-dependence of apical to basolateral permeation rates of cyclosporin (CyA) through Caco-2 cell monolayers. CyA was given into the apical compartment at the indicated concentrations, at 15 min intervals a 200 μl aliquot was taken from the basolateral compartment and the drug concentration was determined. (▲) Measured values; (□) calculated permeation by diffusion; (○) calculated active transport; (△) fitted net amount of drug permeated = difference between diffusion and active transport.

transport system operating in the basolateral to apical direction. The permeation rates of the extracellular marker compound PEG-4000 were similar in both directions, indicating that the tight junctional integrity of the cell monolayers was not affected in these experiments.

Preincubation of the cells with 10 μM of the non-immunosuppressive cyclosporin SDZ PSC 833 for 30 min before administration of [^{3}H]-CyA into the apical compartment of the diffusion chamber or coadministration of 10 μM or 20 μM SDZ PSC 833 together with 0.1 μM [^{3}H]-CyA resulted in increased apical to basolateral permeation rates of CyA through the cell monolayers (Figure 3).

When the cells were preincubated from their apical side with other substrates of p-glycoprotein, such as vinblastine or daunomycin, or when the p-glycoprotein substrates were coadministered together with [^{3}H]-CyA, the apical to basolateral permeation of CyA was also increased (Figure 4a,b). In contrast, basolateral to apical permeation of CyA showed a dose-dependent decrease after preincubation of the cells with vinblastine (Figure 4c).

In a control experiment the permeation of the p-glycoprotein substrate daunomycin was measured. Transport of 0.1 μM [^{3}H]-daunomycin through Caco-2 monolayers from the basolateral to the apical compartment showed a decrease of the permeation rate in the presence of 10 μM CyA (Figure 5), supporting the hypothesis that CyA interacts directly with p-glycoprotein in the Caco-2 cell system. When apical to baso-

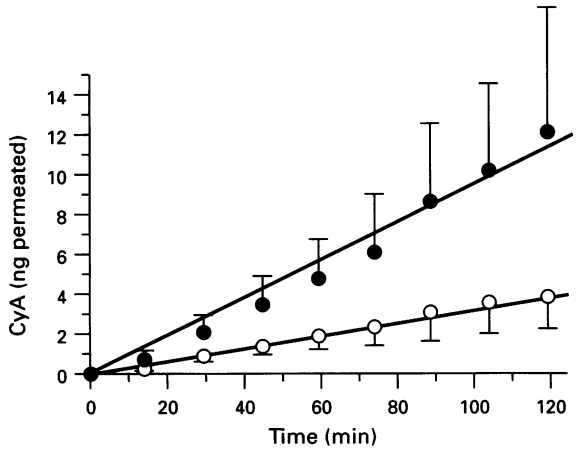

Figure 2 Apical to basolateral (○) and basolateral to apical (●) permeation of 0.1 μM [^{3}H]-CyA ($n=12$). Vertical lines show s.e.mean.

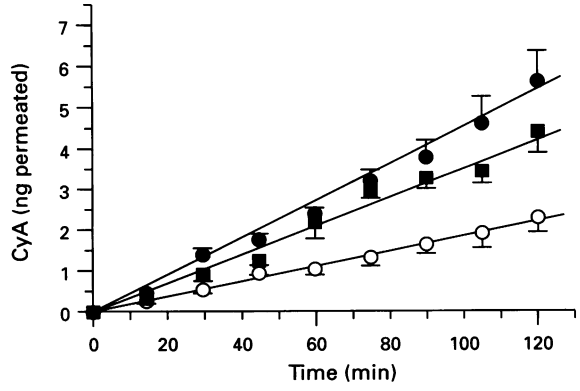

Figure 3 Apical to basolateral permeation of 0.1 μM [^{3}H]-CyA in the absence (control) and in the presence of 10 μM (■) and 20 μM (●) SDZ PSC-833. SDZ PSC-833 was given simultaneously with CyA into the apical compartment ($n=12$). Vertical lines show s.e.mean.

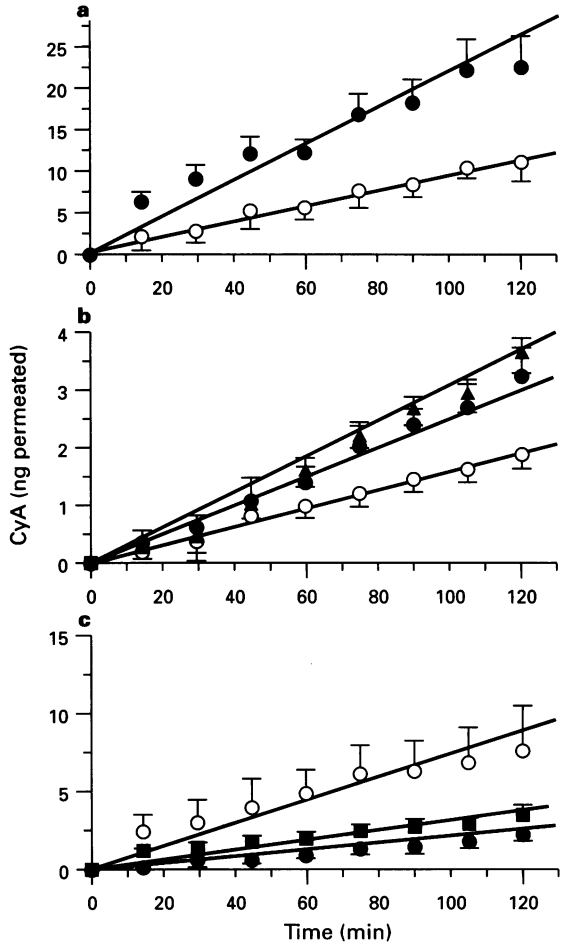

Figure 4 (a) Apical to basolateral permeation of 0.1 μM [^{3}H]-CyA after preincubation of the cells with 10 μM vinblastine (●). The cells were incubated for 30 min from their apical side, then the medium was changed and the permeation of CyA was determined. Control values were determined without preincubation (○); ($n=12$). (b) Apical to basolateral permeation of 0.1 μM [^{3}H]-CyA in the absence (○) and in the presence of 50 μM vinblastine (▲) or daunomycin (●); ($n=12$). (c) Basolateral to apical permeation of 0.1 μM [^{3}H]-CyA without (○) or after preincubation of the monolayers from the apical side with 10 μM (■) or 50 μM (●) vinblastine; ($n=12$). Vertical lines show s.e.mean.

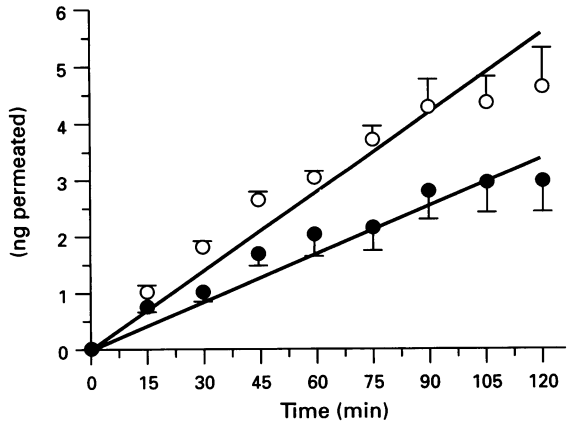

Figure 5 Basolateral to apical permeation of 0.1 μM [^{3}H]-daunomycin in the absence (○) and presence (●) of 10 μM CyA ($n=6$). Vertical lines show s.e.mean.

lateral permeation and basolateral to apical permeation at a concentration of 0.1 μM daunomycin were investigated, the differences in permeation rates were qualitatively similar to those described for CyA (data not shown).

Since all the kinetic data point to a direct involvement of p-glycoprotein with the overall permeation of CyA, the presence of the carrier protein in the cell culture system was assessed by molecular identification with Western blot analysis by use of a monoclonal anti-p-glycoprotein antibody. Sublines of murine monocytic leukaemia P388 cells, one parental (Par-P388) and one multidrug-resistant (MDR-P388), were used as negative and positive controls. Western blots resulted in a distinct labelling of protein in the molecular weight range of 170,000 that paralleled the labelling of the positive control cells, thus being indicative of the presence of p-glycoprotein in the used Caco-2 cells (Figure 6).

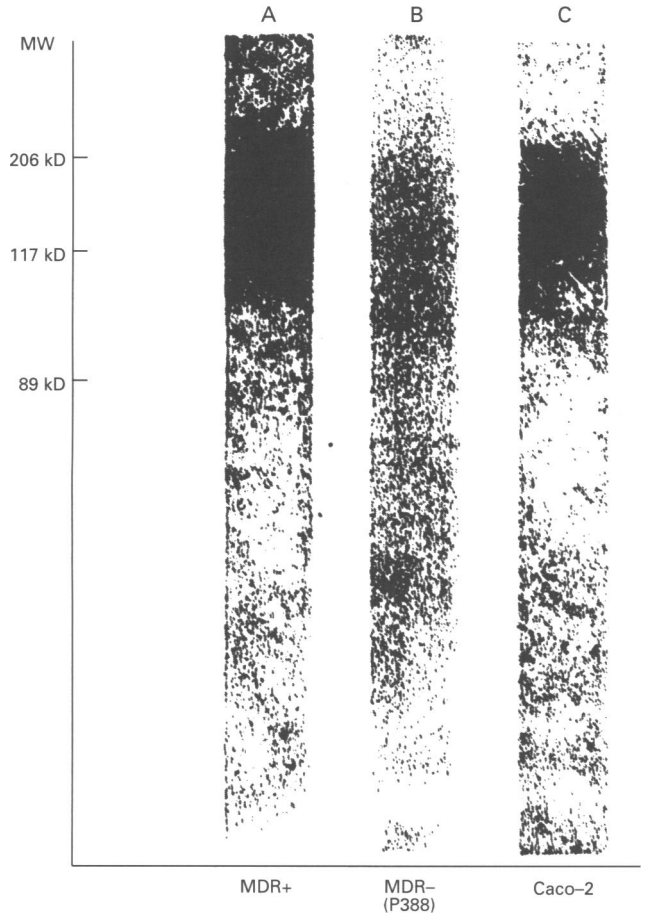

Figure 6 Western blot detection of p-glycoprotein in a positive multidrug-resistant subline of murine monocytic leukaemia P388 cells (MDR+P388; lane A), and in Caco-2 cells (lane C). p-Glycoprotein cannot be detected in a negative subline of the leukaemia cells (Par-P388; lane B).

During the intubation study with human volunteers no drug related adverse events were noted. The administrations were well tolerated and clinically significant changes in laboratory measurements (including creatinine clearance) were not observed. The bioavailability of CyA at all locations was determined and a comparison was made using the absorption from the stomach as a reference. The absorption efficiency gradually decreased from the stomach to the colon, i.e. the relative fraction absorbed was 100% in the stomach, 47% in the jejunum/ileum and 2% in the colon (Table 1). In parallel the coefficients of variation of the blood drug concentrations over time curves markedly increased from the upper to the lower GI-tract (17% in the stomach, 65% in the jejunum/ileum and 196% in the colon). t_{max} was similar after administration into the stomach and jejunum/ileum (1.0 and 1.5 h, respectively), whereas it was significantly delayed after colonic administration (2.2 h).

In previous studies the extent of mRNA expression of p-glycoprotein was measured over the total length of the GI tract (Fojo *et al.*, 1987). mRNA levels progressively increased from the stomach to the colon: low levels in the stomach, intermediate in jejunum and high levels in colon. When the AUCs of CyA after administration at distinct locations in the present study were plotted versus the extent of p-glycoprotein expression found in the different regions of the GI-tract (Figure 7) a marked correlation was found ($r = -0.994$), suggesting that CyA absorption in man may be modulated by p-glycoprotein.

Discussion

Absorption of cyclosporin A in the gastrointestinal tract is highly variable (Lindholm *et al.*, 1988). Several factors, such as presystemic metabolism, poor solubility in the intestinal lumen

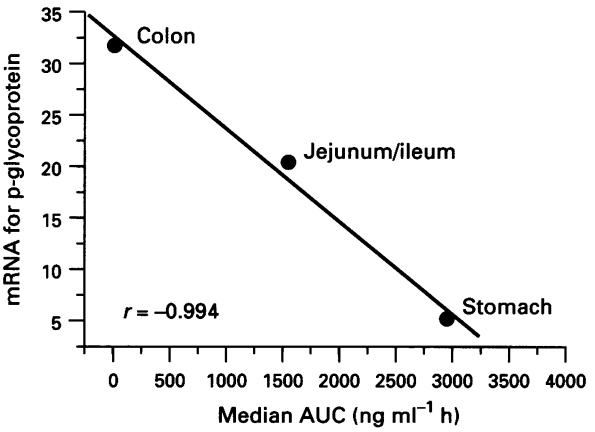

Figure 7 Correlation of CyA blood level AUCs after administration at distinct locations of the GI-tract and mRNA expression of p-glycoprotein in the respective tissue.

Table 1 Pharmacokinetic parameters of cyclosporin A after its administration at different locations in the GI-tract

	Stomach	*Jejunum/ileum*	*Colon*
AUC (0–32 h) (ng ml^{-1} h)	2980 (17.4)	1570 (53.1)*	61 (198.3)*
Rel. fraction absorbed	100	47 (65.3)	2 (196.2)
t_{max} (h)	1.5 (0.3–2.0)	1.0 (0.5–3.0)	2.2 (0.3–5.0)#
C_{max} (ng ml^{-1})	849 (13.6)	489 (60.9)	56 (158.4)*

The values are given as medians of 12 experiments; the numbers in parentheses represent the % coefficients of variation. *Significantly different from administration to the stomach ($P<0.05$); #significantly different from administration to the stomach ($P<0.056$).

and low absorption rates may contribute to this variability. The present transport and inhibition studies suggest that CyA absorption may also be influenced by the presence of p-glycoprotein located in the apical membrane of enterocytes. Caco-2 cells, a widely established *in vitro* system for the investigation of intestinal absorption processes, served as a model in our studies. They represent a cell line originally derived from a colon carcinoma. As previously discussed (Audus *et al.*, 1990), the selection of the cell line becomes particularly important to mimic successfully a biological barrier. Therefore, Caco-2 were used, since they exhibit the functional characteristics of cells of the lower small intestinal tract (Pinto *et al.*, 1983; Wilson *et al.*, 1990; Hidalgo & Borchardt, 1990a,b). The cells used by us showed a directed transport of taurocholate and phenylalanine and expressed a high activity of alakaline phosphatase at confluency. The present study showed both on a molecular and on a functional level the expression of p-glycoprotein, thus confirming previous functional studies (Karlsson *et al.*, 1993; Hunter *et al.*, 1993). Recent findings imply a high expression of p-glycoprotein in the lower GI-tract (Fojo *et al.*, 1987). Both in colon and the lower small intestinal tract high levels of p-glycoprotein have been detected (Thiebaut *et al.*, 1987). Thus, considering their origin, it is not surprising that Caco-2 cells also possess high levels of p-glycoprotein.

Uptake of CyA into the cells occurs by passive diffusion, an observation that has also been made in other epithelial cell types, such as renal tubular cells or hepatocytes (Ziegler *et al.*, 1988; Schramm *et al.*, 1995). Secretion, however, occurs at least partially by an active system. Obviously, CyA is a transported substrate of p-glycoprotein, which is involved in the regulation of the permeation across Caco-2 cell monolayers by modulating the otherwise passive diffusion of CyA through the cells. In that system at low concentrations the activity of p-glycoprotein seems to be rate limiting for the overall permeation of CyA. The calculated kinetic parameters ($K_M = 3.8$ μM, $J_{max} = 6.5$ picomol min^{-1} per filter) were comparable to those found for the active transport component in other cell types expressing p-glycoprotein ($K_M = 8.4$ μM, $J_{max} = 40$ picomol min^{-1} mg^{-1} protein (Saeki *et al.*, 1993)). At higher concentrations the transport system back into the apical lumen becomes saturated and the diffusion rate through the cells is the overall limiting factor. Thus, the profile of the concentration-dependent diffusion rates can be explained. This finding also correlates with the *in vivo* observation of increasing rate and extent of cyclosporin A absorption with increasing doses (Ueda *et al.*, 1984). We are aware that the calculated values give only estimates, because at low concentrations the profile of the measured curve cannot exactly be described by the difference of a term for diffusion and an active transport process. A certain time lag has to be considered between uptake and subsequent secretion. In addition, the intracellular concentration might be different from the one, which is used in the term for the diffusion [i.e. extracellular concentration]. Nevertheless our assumption is justified by the inhibition experiments and observations made in other epithelial cell systems: our own experiments with intact renal tubules from killifish demonstrated slow uptake of a CyA derivative into tubular cells and a rapid secretion into the tubular lumen, which could be inhibited by p-glycoprotein substrates, but not by substrates of the 'classic' renal organic anion and cation transporters (Schramm *et al.*, 1995).

The findings of the intubation study and the remarkable correlation between CyA absorption and p-glycoprotein over the GI-tract emphasize the clinical relevance of our *in vitro* findings to the overall pharmacokinetics of CyA. The differences in the fraction absorbed and that reaching maximum blood levels at later time points after administration in the lower GI-tract support our hypothesis. Since just passive diffusion does not explain the decreased bioavailability after colonic administration, additional factors have to be considered for the absorption process. Besides the influence exerted by gastrointestinal juices such as bile (Venkatamaranan, 1985; Mehta *et al.*, 1988), presystemic metabolism could also contribute to the variability in CyA absorption (Vickers *et al.*, 1992; Webber *et al.*, 1992). The previous finding of a higher CyA metabolism in duodenum as compared to colon (Webber *et al.*, 1992) is not compatible with our results.

There is also increasing *in vitro* and *in vivo* evidence, that p-glycoprotein interactions with CyA may influence the tissue distribution of many drugs, e.g. of lipophilic steroids (van Kalken *et al.*, 1993), digoxin (Ito *et al.*, 1993) or colchicine (Speeg & Maldonado, 1994). Therefore, potential overlapping specificities at the p-glycoprotein transport level in epithelial tissues should be considered when cyclosporins are given together with other drugs. Several clinical studies have demonstrated such interactions: for example, a clinical trial with etoposide revealed a marked effect of cyclosporin upon the pharmacokinetics of etoposide with a doubling of the area under the concentration time curve as a result of both decreased renal and nonrenal clearance, presumably via interactions with p-glycoprotein (Lum *et al.*, 1993).

In summary, the data suggest that the intestinal absorption of cyclosporins is modulated by the presence of p-glycoprotein in the apical enterocyte membrane. Considering the expression of p-glycoprotein in other epithelial cells like kidney tubular cells, hepatocytes and the blood brain barrier, its contribution to the overall pharmacokinetics of cyclosporins should be re-evaluated.

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