Human Jejunal Permeability of Cyclosporin A: Influence of Surfactants on P-Glycoprotein Efflux in Caco-2 Cells

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Purpose. The purpose of this work was to determine the jejunal permeability of cyclosporin A (CsA) in humans and whether formulation variables modulate the effects of P-glycoprotein (P-gp) on the permeability of CsA in Caco-2 cells.

Methods. A solution containing CsA, phenylalanine, propranolol, polyethyleneglycol (PEG) 400, and PEG 4000 was perfused through a 10-cm jejunal segment in 12 subjects. Caco-2 transport studies were performed using previously reported methodology.

Results. The mean P_{eff} (±SD) of CsA in humans was 1.65 (0.53). The mean permeabilities for phenylalanine, propranolol, and PEG 400 were 4.54 (2.39), 2.90 (1.28), and 0.83 (0.51) $\times 10^{-4}$ cm/s, respectively. The presence of surfactants significantly decreased the permeabilities of CsA in both directions in Caco-2 cells.

Conclusions. The results suggest that the effects of surfactants *via* micellar solubilization and inhibition of P-gp efflux on CsA transport in Caco-2 cells are significant. CsA can rightly be classified as a low solubility-high permeability Class II BCS drug and its highly variable absorption from Sandimmune® oral formulations is the result of poor dissolution characteristics.

KEY WORDS: intestinal permeability; P-glycoprotein; cyclosporin A; biopharmaceutic classification system; Caco-2 cell culture.

INTRODUCTION

Cyclosporin A (CsA) is a neutral, hydrophobic cyclic peptide consisting of 11 amino acids. CsA has been widely used as a potent immunosuppressant to prevent organ rejection after kidney, liver, and heart allogeneic transplantation. It has also been used in the treatment of autoimmune diseases, such as rheumatoid arthritis, uveitis, and psoriasis. Because of its poor solubility in water, ~4 μg/mL (1), intravenous and oral dosage forms of CsA contain surfactants. The pharmacokinetics of Sandimmune®, an oral formulation of CsA, in healthy volunteers have been reported (2,3) and can be described by a two-compartment model with zero-order absorption. Although CsA has a high octanol–Ringer's partition coefficient of 991, the absorption from the gastrointestinal tract is incomplete and variable. The oral bioavailability is approximately 30% from a Sandimmune® oral formulation with wide inter- and intrasubject variability (4). Several factors have been suggested as possible determinants of the low and variable oral bioavailability of CsA. These include a low permeability to the intestinal membrane, poor dissolution characteristics, extensive metabolism by cytochrome P-450 3A4 (CYP3A4) in both liver and gut (5,6), effect of P-glycoprotein (P-gp)-mediated drug efflux (7), and the influences of intake of food and concomitant medications (8,9). Some reports suggest that there might be an absorption window for CsA in the upper small intestine (10). However, the mechanism of absorption of CsA, its intrinsic permeability to the intestinal membrane in humans and the underlying cause of its highly variable bioavailability have not been fully addressed.

Intestinal permeability is a key factor in determining overall absorption of orally administered drugs. The determination of effective intestinal permeabilities of drugs in humans has been facilitated by recent innovations such as the Loc-I-Gut® segmental jejunal perfusion system (11). The advantages of this perfusion system include minimizing leakage of proximal and/or distal contents into the test segment and of drug perfusate out of it as well as allowing perfusion to be carried out at physiologic flow rates with a segment of known length (10 cm). Furthermore, this system has been shown to provide higher and more consistent recovery and a mass balance that is expected from the disappearance of drug from the intestinal lumen and its appearance in plasma (11,12). In this report we describe the permeability of CsA in human subjects estimated from segmental jejunal perfusion studies. Consistent with previously reported standard procedures, the present permeability studies included marker compounds such as propranolol, phenylalanine, and polyethyleneglycol (PEG) 400 that are absorbed to varying degrees and by different pathways (13,14), and were used as reference compounds in order to determine both intra-subject and inter-subject variability.

Caco-2 cell cultures have been widely used to investigate absorption mechanisms of several classes of drugs (15,16). These studies indicate that the evaluation of effects of formulation variables such as surfactants on P-gp efflux mechanisms in Caco-2 cells may provide useful information regarding CsA absorption characteristics in humans. Thus, in this report we also describe the results of the effects of surfactants and inhibitors of P-gp on permeability of CsA in Caco-2 cells. The objective of these studies involves the evaluation of the intrinsic permeability of CsA in the two models and to elucidate the role of P-gp efflux mechanisms in determining overall absorption of the drug. A secondary and important objective of these studies is to extrapolate these findings to absorption characteristics in humans in an attempt to explain the highly variable oral bioavailability of CsA.

MATERIALS AND METHODS

Materials

USP grade *l*-phenylalanine was obtained from Twin Laboratories Inc. (Ronkonkoma, NY, USA). Propranolol

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HCl (SoloPak®, IV injection) was purchased from SoloPak Laboratories Inc. (Elk Grove Village, IL, USA). (mebmt-β-^{3}H) CsA with a specific activity of 6 Ci / mmol was from Amersham Company. CsA (Sandimmune® IV solution) was purchased from Sandoz (East Hanover, NJ, USA). Cremophor EL® (polyoxyethylene 35 castor oil) and Cremophor RH40® (polyoxyethylene 40 castor oil) were obtained from BASF (Mount Olive, NJ, USA). d-alpha tocopheryl polyethylene glycol 1000 succinate (TPGS®) was from Eastman. Dulbecco's modified Eagle's medium, D-glucose, fetal bovine serum, nonessential amino acids, L-glutamine, Na-pyruvate, and penicillin (100 U/mL)/streptomycin (100 μg/mL) was obtained from Gibco BRL (Grand Island, NY, USA). PEG 400, 1000, 1500, and 4000 were obtained from Union Carbide Chemicals and Plastic Company, Inc. (Danbury, CT, USA). EcoLite(+) scintillation cocktail was from ICN Company. Midazolam (intravenous injection) was purchased from Roche Pharmaceuticals (Nutley, NJ, USA). Fentanyl (intravenous injection), xylocaine and simethicone were USP or NF grade. Tris hydrochloride, glycerol, ethylenediaminetetraacetic acid, phenylmethylsulfonyl fluoride, MES, HEPES, quinidine, progesterone, chlorpromazine HCL, and verapamil HCL were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Mannitol, $NaH_2PO_4 \cdot H_2O$, Na_2HPO_4, glucose, KCl, and NaCl of USP or NF grade were used to prepare perfusate solutions and were purchased from Spectrum Chemical Mfg. Corp. (Gardena, CA). CsA (batch 92359.02) for preparation of standards for high-performance liquid chromatography (HPLC) and for permeability studies in rats and CaCo-2 cells was obtained from Sandoz (East Hanover, NJ). HPLC standards for *l*-phenylalanine and *dl*-propranolol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All solvents used in the assays were of HPLC grade. All other chemicals were either HPLC or analytical grade.

Intestinal perfusions in humans were performed with a multi-channel tube designed for study of absorption and secretion in the small intestine in humans (Loc-I-Gut™, Nolato AB, Sweden; Ref. 11). It contains six channels and two latex balloons separated by 10 cm to create the segmental perfusion site. Presterilized cell tissue culture inserts with Cyclopore® membrane (25 mm diameter and 3-μm pore size) as well as tissue culture treated polystyrene 6-well plates were from Falcon (Lincoln Park, NJ, USA).

Caco-2 Cells

Caco-2 cells (ATCC HTB37, Manassas, VA, USA) were routinely maintained in Dulbecco's modified Eagle's medium containing 4.5 g/l D-glucose, 10% fetal bovine serum, 1% nonessential amino acids, 1% L-glutamine, 1 mM Na-pyruvate, 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were grown in 100-mm tissue culture petri dishes and passaged every 5 days at a split ratio of 1:5 and all cells were maintained in an atmosphere of 5% CO_2 and 90% relative humidity at 37°C. The medium in the cell culture chambers was changed daily. Cells were used at passage numbers 37 to 45. Before reaching confluency, the cells were subcultured with trypsin–ethylenediaminetetraacetic acid (0.25%, 0.02%) and seeded at a density of 3×10^5 cells per 25-mm culture insert (3.0 μM pore size). Cells were used between 2 to 3 weeks after seeding on to the inserts. The culture medium was changed every other day for the first two weeks and once a day thereafter. Cell integrity was evaluated by monitoring Transepithelial Electrical Resistance (TEER) using an EVOM® Epithelial Voltohmmeter equipped with "chopstick" electrodes (World Precision Instruments, Sarasota, FL, USA).

Subjects

All subjects gave written informed consent to participate in the study that followed the tenets of the Declaration of Helsinki promulgated in 1964 and was approved by the Institutional Review Board at the University of Michigan Medical Center. All subjects were between the ages of 21–31 years and were judged healthy based on medical history, physical examination, and laboratory tests prior to the study period.

CsA Transport Studies with Caco-2 Cells

Cells were used between 2 to 3 weeks after seeding on to the inserts. Inserts containing the Caco-2 monolayers were positioned in six-well plates such the outer surface of the inserts (basolateral side) was immersed in the transport medium. The general procedure for studying transport phenomena of CsA in Caco-2 cells was as follows: All solutions and buffers were pre-warmed at 37°C. The inserts containing Caco-2 cells were washed twice with phosphate buffer (pH 7.4) before the transport study. The inserts were then immersed into HEPES buffer, pH 7.4, with 2 mL on the top of the membrane and 2.5 mL on the bottom. After equilibration for 30 min, triplicate measurements of TEER values of the inserts were undertaken. For the apical to basolateral side transport studies (AP to BL), 1.5 ml of the test CsA transport solution was placed on top of the insert (apical side of membrane). CsA-free transport solution of an identical composition (2.5 mL) was placed on the bottom (basolateral side of the membrane). Basolateral-to-apical side transport studies (BL to AP) were performed by reversing the placement of the test CsA and the CsA-free solutions. The solution from the appropriate receiver compartment was collected *in toto* at predetermined intervals for CsA assays, and was replaced by an equal volume of fresh CsA-free transport solution. Test transport solutions comprised 4.0 μg/mL CsA containing trace amount of ^{3}H-CsA and varying amounts (0-2%w/v) of either Cremophor EL® or Cremophor RH40® or TPGS® in pH 7.4 HEPES buffer. Transport studies to determine the effects of P-gp inhibitors on CsA transport were conducted using solutions containing 0.4 μg/ml or 4 mg/ml CsA with trace amount of ^{3}H-CsA, and 0-100 μM of either verapamil, progesterone, quinidine or chlorpromazine in pH 7.4 HEPES buffer. At the end of the experiment, TEER values were again measured in triplicate to establish effects of surfactants on monolayer integrity. Non-specific binding of CsA to the surface of the cell culture device was determined by soaking the device in a solution containing 2% Cremophor EL® followed by mixing with EcoLite(+) that allowed dissolution of the polystyrene treated plate and ascertaining that CsA recovery was complete. Appropriate amounts of EcoLite(+) cocktail were added to all transport sample solutions as well as pipet-tips, vortex mixed and assayed using a Beckman LS 6000 SC scintillation counter.

Human Jejunal Perfusion Studies

Subjects were admitted to the General Clinic Research Center at the University of Michigan Medical Center on the

day of the study at 7 am and fed a standard breakfast over the next one and a half hours. A 5-mL blood sample was obtained to determine the concentration of testosterone or estradiol. The subjects remained fasted under supervision for the duration of the study, approximately 14 h. The intubation and placement of the perfusion tube in the upper jejunum was performed according to the procedure described previously (11). The jejunal segment was first rinsed with 120–180 mL of normal saline prewarmed to 37°C. The segment was then perfused with the drug-containing solution pre-warmed to 37°C at a rate of 3 mL/min for 120 min. Sandimmune® intravenous solution was used to prepare CsA perfusate solutions since it was the only drug form of CsA approved for human use that was available to us. Further, an additional 0.2% (w/v) of Cremophor EL® was incorporated in the perfusate solution in order to prevent adsorption of CsA to the tubing. Thus, the perfusate solution contained 4.2 μM (5 μg/mL) CsA, 0.2% Cremophor EL, 12.3 mM PEG 400, 1.4 mM PEG 4000, 3.4 μM *dl*-propranolol, 60.5 μM *l*-phenylalanine, 10 mM *d*-glucose, 5.4 mM KCl, 35.1 mM *d*-mannitol, 45 mM NaCl, 49 mM $NaH_2PO_4 \cdot H_2O$, and 21 mM Na_2HPO_4. The final osmolality and pH of the perfusate solution was adjusted to 290 mOsm/L and 6.5, respectively. The fluid leaving the intestinal segment was collected on ice every 10 min by gravity drainage. During the entire study a vacuum pump (4 psi) was used to facilitate continuous aspiration of accumulated gastric and intestinal fluids in order to relieve bloating effects and also to minimize their possible leakage into the perfused segment. All syringes and samples were immediately weighed and the samples were stored at –20°C until assay. All subjects remained fasted and in a recumbent position until completion of the perfusion study.

Drug Analysis

The concentrations of CsA, *l*-phenylalanine, *dl*-propranolol, PEG 400, and PEG 4000 in human perfusion study samples were assayed by high performance liquid chromatography (HPLC) methods. The HPLC system consisted of a Waters interface module system, a Waters WISP™ 712 autosampler, a Waters 410 differential refractometer, a Waters 470 fluorescence detector, a Waters 996 photodiode array detector, two Waters HPLC 515 pumps, a Waters 2.15 Millenium® chromatography manager system (Waters Corporation, Milford, MA, USA), and an Eppendorf CH-30 column heater and TC-50 temperature controller (Eppendorf, Westbury, NY, USA). CsA was assayed using previously reported HPLC methods (17). Prior to extraction of CsA from perfusate samples, 2% Tween 80 was incorporated in order to prevent adsorption losses. CsA was extracted from the perfusate by the following method. One ml of sample or standard was mixed with 4 ml of ethyl acetate. It was then vortexed at 2000 rpm on a VXR shaker (Staufen, Germany) for 10 min. After centrifuging for 10 min at 885 *g*, an amount of 3 mL solution from the ethyl acetate layer was transferred using glass pipettes and evaporated to dryness under nitrogen gas at the room temperature. The residue was reconstituted with 200 μL of 80% acetonitrile water (v/v) solution, vortexed, and filtered through a 0.45-μm syringe filter. An amount of 20 μL of reconstituted solution was then injected into HPLC for assay (17). The recovery ratio of this extraction method was 85%. Propranolol was analyzed using a modification of a previously reported method (18). The modification involved the use of mobile phase containing (16:4:80, v/v) of methanol, tetrahydrofuran, and a buffer solution containing 1 g of 1-heptanesulfonic acid sodium salt, 15 mL of triethylamine and 1000 ml of HPLC grade water. The pH of the buffer solution was adjusted to 2.3 with concentrated phosphoric acid, filtered and degassed before use. Analyses of PEG 400 and PEG 4000 were conducted using previously described methods (11). CsA concentrations in Caco-2 cell studies were determined by scintillation counting.

Data Analysis

Permeability Coefficients in Caco-2 Cells

The effective permeability value (P_{eff}) was calculated based on Fick's law:

$$P_{eff} = (dQ/dt)/(A * C_o) \quad (1)$$

where dQ/dt is the flux across monolayer (dpm CsA/min); A is the surface area of membrane (4.91 cm^2); and C_0 is the initial drug concentration (dpm CsA/mL).

Estimation of Effective Permeability Coefficients in Perfusion Studies

The effective permeability of the intestinal segment can be calculated at steady state, typically achieved after about 30-40 min of perfusion, using mass balance on the perfusion system as described previously (11).

$$\frac{dM}{dt} = Q(C_{in} - C_{out}) = A \cdot P_{eff} \cdot C_{out} \quad (2)$$

where C_m is the inlet concentration of the drug and C_{out} is the outlet concentration. C_{out} was assumed to be equal to the concentration in the perfused jejunal tube segment because of the assumption of complete mixing with the mixing tank model (11). P_{eff} is the effective permeability, Q is the volume flow rate of the intestine and A ($= 2\pi RL$) is the area of the mass transfer surface of the jejunal segment. Rearranging Eq. (1) in terms of P_{eff} results in

$$P_{eff} = \frac{Q}{A}\left(\frac{C_{in}'}{C_{out}'} - 1\right) = \frac{Q}{2\pi RL}\left(\frac{C_{in}'}{C_{out}'} - 1\right) \quad (3)$$

C_{out}'/C_{in}' is the fractional concentration of the drug adjusted for the water transport determined using PEG 4000 (a non-absorbable marker) and is calculated as

$$\frac{C_{out}'}{C_{in}'} = \frac{[Drug]_{out}}{[Drug]_{in}} \times \frac{[PEG4000]_{in}}{[PEG4000]_{out}} \quad (4)$$

L is the length of the perfused segment (10 cm) and R is the apparent individual radius of the segment. The individual segmental radius was calculated using the segmental volume estimation as described earlier (11).

After calculation of effective permeability coefficients from various human subjects with equation 3, a Z-score test using three standard deviations as the exclusion criteria was applied for outlier detection as in the previous study (11). The effective permeability coefficients obtained after excluding outliers were then normalized to a fixed segmental radius of

1.75 cm. The mean of the normalized effective permeabilities was then used to calculate fraction of drug absorbed.

Residence Time Distribution (RTD) Analysis

A noninteger mixing tank model was used to analyze the residence time distribution as described previously (11). The mean residence time and number of tanks in the perfused segment were predicted from RTD analysis. The fractional concentration curve (F curve) was calculated from the concentration of PEG 4000 by fitting the following equation:

$$F = 1 - \left(\frac{r}{r-1}\right)^{n} e^{\frac{n+r}{rt}t} - e^{\frac{n+r}{t^*}t} \sum_{i=1}^{n} \left(1 - \left(\frac{r}{r-1}\right)^{n-i+1}\right) \frac{1}{(i-1)!} \left(\frac{n+r}{t^*}t\right)^{i-1} \quad (5)$$

where F is the fraction of PEG 4000, n is the number of tanks, r is the fraction tank, t is the time, and t^* is the mean residence time. The time (t) was calculated by subtracting the residence time in the perfusion tubes, to account for the tube hold-up time, and by using the mid-time during the 10-min sampling collection period.

Statistical Analysis

Statistical analysis was performed using two-way analysis of variance with Microsoft® Excel 97.

RESULTS

Effects of Surfactants on CsA Transport in Caco-2 Monolayers

The application of up to 2% (w/v) of Cremophor EL®, Cremophor RH40®, or TPGS® to either the apical or basolateral side of Caco-2 monolayers for 2 h did not affect TEER values significantly. The results suggest that the integrity of the monolayers was maintained in the presence of the surfactants. The effects of varying concentrations of surfactants on the directional transport of CsA from perfusate solutions containing 4 μg/mL CsA are shown in Figs. 1–3. The results indicate that in the absence of surfactants, the permeability of

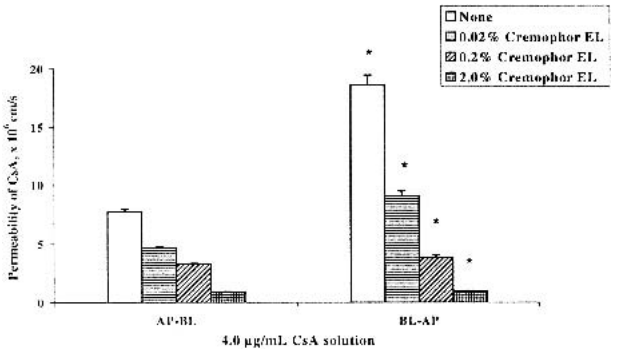

Fig. 1. Effect of Cremophor EL® concentration on directional permeability coefficient (mean ± SD) of cyclosporin A in Caco-2 monolayers. n = 3. (BL-to-AP permeabilities significantly different from each other; $p < 0.05$.)

CsA in the BL to AP direction is twice as high as that in the AP-to-BL direction, suggesting net secretion. The permeability of CsA decreased significantly with increasing surfactant concentration in both AP-to-BL and BL-to-AP directions. The magnitude of the decrease in permeability in either direction was in the order Cremophor EL® ≥ Cremophor RH40® >> TPGS®.

Effects of P-gp Inhibitors on CsA Transport in Caco-2 Monolayers

The effects of quinidine on the directional permeability of CsA in Caco-2 monolayers at two different concentration ratios are shown in Figs. 4 and 5. It is seen that when the molar ratio of quinidine to CsA is low (3; Fig. 4), its effect on CsA transport in either direction is not appreciably different than the control. However, increasing the molar ratio to 300 (Fig. 5) resulted in a substantial enhancement in the transport of CsA in the AP-BL direction and a significant reduction in its transport in the BL-to-AP direction. These results appear to be consistent with previous findings for a major role of P-gp efflux mechanisms in determining CsA transport in Caco-2 cells. Figure 6 shows a comparison of the enhancement of CsA permeability in the AP-BL direction in the presence of the P-gp inhibitors quinidine, verapamil and proges-

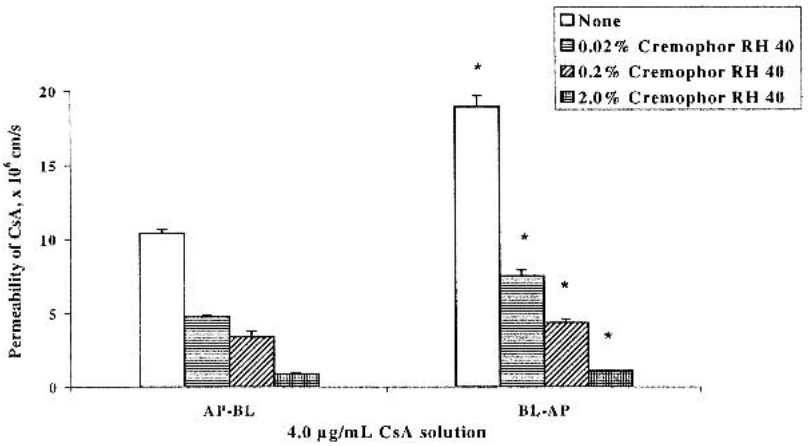

Fig. 2. Effect of Cremophor RH 40® concentration on directional permeability coefficient (mean ± SD) of cyclosporin A in Caco-2 monolayers. n = 3. (BL-to-AP permeabilities significantly different from each other; $p < 0.05$.)

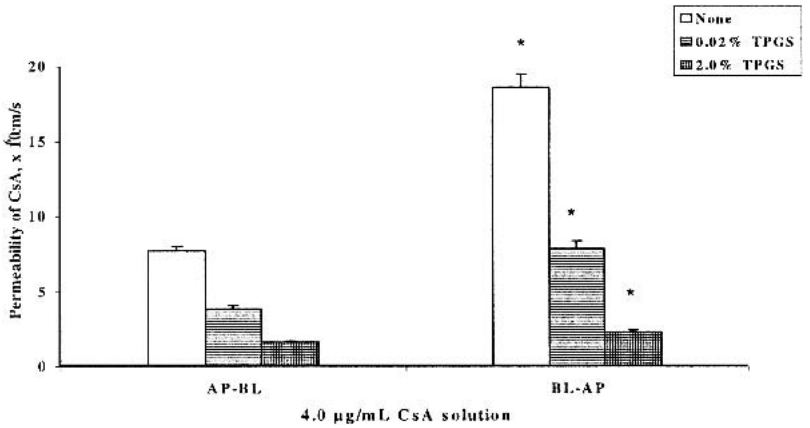

Fig. 3. Effect of TPGS® concentration on directional permeability coefficient (mean ± SD) of cyclosporin A in Caco-2 monolayers. n = 3. (BL-to-AP permeabilities significantly different from each other; $p < 0.05$.)

terone, at a CsA to inhibitor molar ratio of 300. The results indicate that the order of inhibition under these conditions was quinidine > progesterone > verapamil.

Human Jejunal Perfusion Studies

RTD Analysis

F curve and outlet concentration vs. inlet concentration of CsA and that of marker compounds showed that steady state was achieved after 30 min. Thus, the *F* value of PEG 4000 was close to unity from 40 to 120 min. The mean residence time of PEG 4000 was estimated to be (mean ± sd) 7.3 ± 5.5 min, the number of tanks 3.4 ± 3.2 and the recovery ratio of PEG 4000 in all 12 subjects was 85% or higher.

Human Jejunal Permeability of CsA

The mean effective permeabilities of CsA and the reference markers phenylalanine, propranolol and PEG 400 estimated using Eq. (3) and normalized to a fixed segmental radius of 1.75 cm are shown in Table I. A comparison of the normalized mean effective permeabilities of phenylalanine, propranolol and PEG 400 in this study using perfusates containing 0.2% Cremophor EL® with previous estimates (11,12) indicated no significant differences suggesting that Cremophor EL® does not influence the permeability of these compounds (Table I).

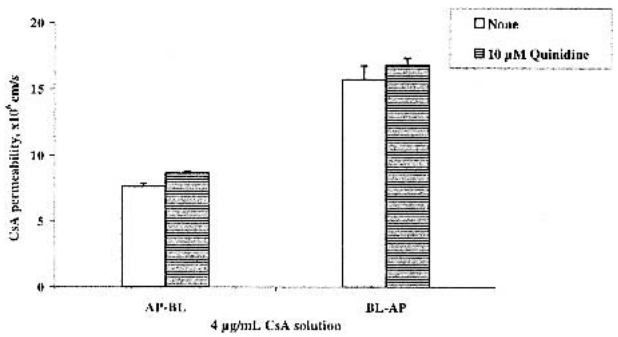

Fig. 4. Effect of 10 μM quinidine on the directional permeability coefficient (av ± SD) of cyclosporin A in Caco-2 monolayers. CsA concentration = 3.34 μM (4.0 μg/mL); n = 3.

DISCUSSION

The dramatic lowering of AP-to-BL (2 to 12-fold) permeabilities of CsA in the presence of surfactants suggests that micellar solubilization of CsA decreases the fraction of "free" unbound CsA that would be available for transport across the monolayer. Thus, apparent permeabilities based on the total CsA concentration would be substantially lower than the intrinsic permeability. Several previous studies examining the effects of nonionic surfactants on transport in cell monolayers have clearly indicated that the AP-to-BL transport of drugs that are P-gp and/or MRP substrates increased at surfactant concentrations at or below the critical micelle concentration (19–22). These studies also showed decreased transport at high surfactant concentrations as a result of micellar inclusion of the drug. The critical micelle concentration (cmc) of Cremophor EL® has been reported to be 0.008%w/v (0.007–0.009% w/v range) in Hanks balanced salt solution at pH 7.4 and 25°C (20), and 0.008% w/v in 0.05 M HEPES buffer, pH 7.0 (23). Further, the free fraction of paclitaxel, a drug that is formulated in a manner similar to CsA, decreased from around 94% at Cremophor EL® concentrations below 0.01% w/v to 46% and 18%, at Cremophor EL® concentrations of 0.0625% and 0.25% w/v, respectively (23). Also, extensive solubilization of tacrolimus in nonionic micelles has been re-

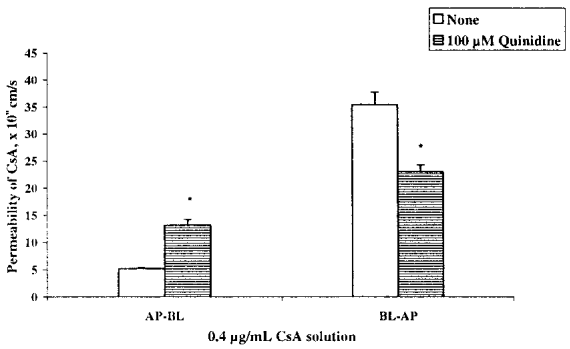

Fig. 5. Effect of 100 μM quinidine on the directional permeability coefficient (av ± SD) of cyclosporin A in Caco-2 monolayers. Cyclosporin A concentration = 0.334 μM (0.4 μg/ml); n = 3. Permeability in the presence of 100 μM quinidine significantly different compared with controls ($p < 0.001$).

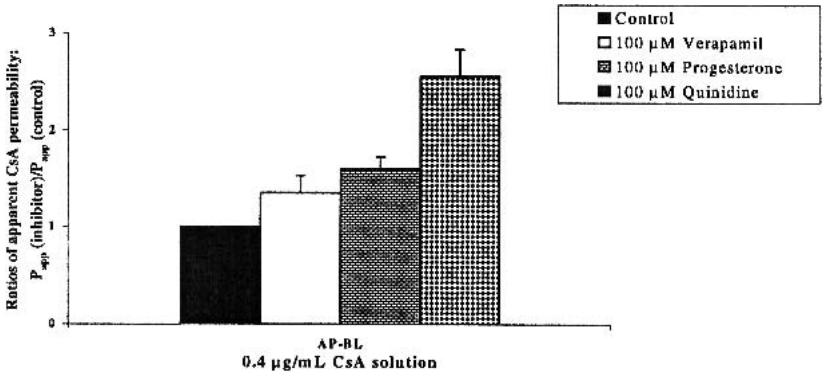

Fig. 6. Comparison of enhancement in AP-to-BL transport of cyclosporin A in Caco-2 monolayers in the presence of various inhibitors. Inhibitor concentration = 100 μM; cyclosporin A concentration = 0.334 μM (0.4 μg/mL); n = 3.

ported (24). It was found that the free fraction of tacrolimus above the cmc of the HCO-60 nonionic surfactant was around 10–15%. The lack of enhancement in apparent AP-to-BL permeability of CsA in our studies may suggest that micellar solubilization effects far outweigh any P-gp inhibitory effects even at the lowest surfactant concentration of 0.02% w/v examined. Indeed, in studies with rhodamine 123 in Caco-2 monolayers, it was shown that the maximal levels of uptake in the presence of various surfactants occurred in the proximity of the respective cmc of the surfactant (22). Because the main objective of these studies was to ascertain surfactant effects at concentrations encountered in commercial CsA formulations, detailed studies such as those described in previous studies (20) were not conducted. Nevertheless, it appears that under the conditions of our Caco-2 monolayer studies, the major effect on AP-BL transport of CsA is caused by lowered CsA activity arising out of micellization that results in lowered apparent permeability coefficients. These effects are expected to operate under *in vivo* conditions as well. The results in Figs. 1–3 also indicate that such micellar solubilization effects by surfactants in the basolateral compartment dramatically lower BL-to-AP transport of CsA in Caco-2 monolayers. It is not possible to ascertain if decreased efflux is due to micellization alone or due to a combination of micellar solubilization and inhibitory effects on P-gp efflux by the surfactants. Previous studies on the effects of nonionic surfactants such as Cremophor EL®, Tween 60 and Pluronic 85, placed on the apical side clearly demonstrated that the BL-AP transport of a variety of P-gp substrates were significantly reduced reflecting an inhibitory effect on P-gp efflux pump (19,21). It was also shown that only monomers affected P-gp efflux and that transport effects observed with surfactant concentrations higher than the cmc were independent of the side of administration.

The results of the Caco-2 transport studies are consistent with the following scenario. In the presence of surfactant concentrations that are much higher than the cmc, any enhancement in CsA transport that might have resulted from inhibition of P-gp efflux mechanisms by surfactant monomers are quite overwhelmed by the much larger negative effect of surfactant micelles on CsA transport. The net result of the two opposing effects is to provide apparent permeability values that are substantially lower than the intrinsic values.

The effective permeability of CsA in human jejunum from perfusate solutions containing 0.2% w/v Cremophor EL® is therefore expected to be lower due to micellar solubilization of CsA in Cremophor micelles. The Caco-2 transport studies suggest that the apparent permeability in the presence of 0.2% Cremophor EL® or Cremophor RH40® could be reduced by a factor of 2 to 4 compared to that in the absence of these surfactants. Thus, the intrinsic permeability of CsA in humans could be substantially higher than the estimated mean normalized effective permeability value of 1.65×10^{-4} cm/s. The fraction dose absorbed calculated using a permeability value of 1.65×10^{-4} cm/s, a mean residence time of 3 h, and an intestinal radius of 1.75 cm was 0.87, suggesting that CsA is a high permeability drug. The implications of the overall effects of surfactants on the permeability of CsA indicate that CsA could be classified as a BCS Class II low-solubility, high-permeability drug (25). The high variability of absorption characteristics from Sandimmune® oral formulations in humans may thus be due to other factors.

Table I. Normalized Mean Effective Permeabilities of Cyclosporin A, and Various Reference Compounds Estimated Using Mean Apparent Individual Segmental Radius and Normalized to a Fixed Radius of 1.75 cm

Compound	P_{eff} ($\times 10^4$), cm/s Ave ± SD	CV	n	P_{eff} ($\times 10^4$), cm/s Ave ± SD[a]
Cyclosporin A	1.65 ± 0.53	0.32	12	–
Phenylalanine	4.54 ± 2.39	0.53	11	4.31 ± 2.11
Propranolol	2.90 ± 1.28	0.44	12	2.70 ± 1.19
PEG 400	0.83 ± 0.51	0.62	12	0.56 ± 0.38

[a] from Ref. 11.

Sandimmune® Oral CsA Formulation vs. Neoral® Oral CsA Formulation

The absorption of CsA from the conventional oral formulation, Sandimmune® is dependent on the presence of bile in the gastrointestinal tract and is highly variable. The mean absolute bioavailability of CsA from Sandimmune® oral formulations in humans has been reported to be around 30% with very high inter- and intra-patient variability (4). The absolute bioavailability of CsA (10 mg/kg) from Sandimmune® oral formulations in healthy volunteers was reported to be 23% in subjects fed a low fat diet and 42% in subjects on a high fat diet (26). To obviate these problems, a novel

microemulsion formulation of CsA was introduced (27). This novel oral formulation, Neoral®, contains corn oil mono-, di-, and triglycerides, Cremophor RH40®, glycerol, and propylene glycol in addition to CsA (100 mg/ml) and ethanol (9.5% w/v). The absolute bioavailability of CsA from Neoral® oral formulations has not been determined yet. However, the relative bioavailability of CsA from Neoral® formulations was compared to that obtained from the Sandimmune® oral formulation in 48 healthy volunteers over the dosage range of 200 to 800 mg (27). It was found that both C_{max} and systemic availability (AUC) were greater for Neoral® at all dose levels. The enhancement in CsA oral bioavailability from Neoral® compared to that from Sandimmune® ranged from 1.74 to 2.39 over the dosage range of 200 to 800 mg. Also, in contrast to dose nonlinearity in CsA pharmacokinetics from Sandimmune® oral formulations, dose proportionality in AUC response was evident from the Neoral® oral formulation. Interestingly, the incidence of double peaks was found to be dramatically reduced at lower doses for the Neoral® formulation compared to Sandimmune® (27). Several studies have also shown that C_{max}, t_{max}, and AUC for CsA after Neoral® oral administration are subject to less variability than with the Sandimmune® oral formulation (28). The absorption of CsA from Neoral® oral formulations in eleven *de novo* liver transplant patients was found to be unaffected by T-tube diversion of bile. Co-administration of food with Neoral® oral formulations was found to decrease AUC by 13%; however, there were no differences between high fat and low fat meals. The expectation of more consistent and superior clinical benefits from Neoral® oral formulations based on its superior pharmacokinetics have been confirmed in several studies. Thus, in renal transplantation, significant reductions in the incidence of acute rejection ($p = 0.016$), multiple rejection, treatment failure in adult recipients ($p = 0.02$), and in the incidence of chronic rejection in pediatric recipients ($p < 0.05$) have been reported (28). More importantly, it was found that there was no increase in the incidence or severity of adverse effects in transplant patients receiving continuous treatment with Neoral® for 2 years (28).

The superior absorption of CsA from Neoral® oral formulations compared with Sandimmune® oral formulations cannot be ascribed to differences in the effects of formulation variables on P-gp efflux mechanisms. Although the two formulations contain different surfactants, this appears to be a reasonable conclusion based on the observations that micellar solubilization effects far outweighed P-gp mediated effects on absorption of CsA in Caco-2 studies. Further, the intrinsic human jejunal permeability of CsA was quite high indicating that intestinal permeability may not be the rate-limiting factor. Thus, other factors, such as dissolution characteristics of CsA from the two formulations, may be involved. Particle size is one of the key parameters that determines drug dissolution from a given formulation. Indeed, the absolute bioavailability of CsA from the Neoral® oral microemulsion formulation (particle size ~40 nm) was determined to be about 3-fold higher than that obtained following oral administration of the Sandimmune® oral formulation (particle size ~860 nm) in rats (29). The role of particle size rather than of surfactant type was also evidenced in their study. Thus, it was found that absolute bioavailabilities similar to that obtained with the Neoral® oral formulation were observed using a microemulsion formulation containing Cremophor EL®, Captex 355®, and Transcutol® comparable in size (~20 nm) to the Neoral® formulation (29). The bioavailability of CsA in rabbits following oral administration of a lecithin-based liposomal formulation (particle size ~60 nm) was found to be similar to that obtained following oral administration of the Neoral® oral formulation (30). The enhanced absorption from formulations with much smaller particle sizes are consistent with the classic use of micronization of dosage forms to improve area under the concentration-time curve (AUC) when compared with the original formulation.

CONCLUSIONS

The presence of 0.2% (w/v) of the noinionic surfactant Cremophor® EL decreased apical-to-basolateral transport of CsA in Caco-2 monolayers about 2- to 3-fold compared with that observed in the absence of surfactant. These effects appear to be modulated mainly *via* micellar solubilization of CsA, thereby reducing thermodynamic activity of CsA and its permeability. It is also possible that the surfactants inhibit P-gp efflux in these models and increase permeability of CsA; however, micellar solubilization effects far outweigh P-gp-mediated increases resulting in a net overall reduction in CsA permeability. Similar reductions in intrinsic permeability of CsA in the presence of surfactants are also expected in human perfusion studies. Thus, the intrinsic CsA permeability in humans is likely to be about two to 3-fold higher than the estimate of 1.65×10^{-4} cm/s from perfusion studies containing 0.2% (w/v) Cremophor® EL. Thus, the intrinsic permeability of CsA is expected to be high and it can rightly be classified as a BCS Class II high permeability-low solubility drug. The highly variable absorption of CsA from Sandimmune® oral formulations in humans appears to be related to the poor dissolution characteristics of the drug.

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