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Transport characteristics of rutin deca (H-) sulfonate sodium across Caco-2 cell monolayers

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Abstract

The aim of this study was to explore potential transport mechanisms of rutin deca (H-) sulfonate sodium (RDS) across Caco-2 cell monolayers. As an in-vitro model of human intestinal epithelial membrane, Caco-2 cells were utilized to evaluate the transepithelial transport characteristics of this hydrophilic macromolecular compound. Bi-directional transport study of RDS demonstrated that the apparent permeability (P_{app}) in the secretory direction was 1.4 ~ 4.5-fold greater than the corresponding absorptive P_{app} at concentrations in the range 50.0 ~ 2000 μM. The transport of RDS was shown to be concentration, temperature and pH dependent. In the presence of ciclosporin and verapamil, potent inhibitors of P-glycoprotein (P-gp)/MRP2, the absorptive transport was enhanced and secretory efflux was diminished. RDS significantly reduced the efflux ratio of the P-gp substrate rhodamine-123 in a fashion indicative of P-gp activity suppression, while rhodamine-123 competitively inhibited the polarized transport of the compound. In conclusion, the results indicated that RDS was likely a substrate of P-gp. Several efflux transporters, including P-gp, participated in the absorption and efflux of RDS and they might play significant roles in limiting the oral absorption of the compound. These observations offered important information for the pharmacokinetics of RDS.

Introduction

Advances in genomics, biotechnology and combinatorial chemistry have led to the discovery of a significant number of therapeutic molecules. For a new chemical entity (NCE) to become a successful drug, there are a multitude of desirable characteristics it should possess: potency to a biological target, selectivity, good stability and physicochemical properties, minimal toxicity and an adequate ADME (absorption, distribution, metabolism and excretion) profile (Balimane et al 2004). Good permeability through intestinal membranes, which leads to adequate systemic absorption, is a property highly desirable in NCEs. A cell model system, such as Caco-2, is commonly utilized during drug discovery and development as a predictive tool to estimate intestinal absorption for drug candidates.

Caco-2 cells originate from a human colorectal carcinoma and spontaneously differentiate on microporous filter membranes into polarized monolayers. They acquire many features of absorptive intestinal cells during culture (Hidalgo et al 1989). Caco-2 cells express several efflux transport proteins that may hamper a drug's absorption, such as P-glycoprotein (P-gp) and members of the multi-drug resistance-associated protein (MRP) family. They belong to the ATP-binding cassette (ABC) superfamily of transport protein and function as energy-dependent efflux pumps, decreasing the transport and cellular uptake of drugs and xenobiotics (Hidalgo & Li 1996). These properties make the system particularly useful as a model for determining a drug candidate's absorption potential, studying the transport mechanism of drugs and elucidating the metabolism of drugs.

Rutin deca (H-) sulfonate sodium, a polysubstituted flavone, possesses very good activity as inhibitor of the complement system of warm-blooded animals and can be used in the therapeutic treatment of certain immunological diseases (Nair & Bernstein 1983). Recent research discovered that this compound possesses potential anti-HIV activity (Mu et al 1998). However, low and variable oral bioavailability of RDS has

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been observed in pharmacokinetic studies in dogs. Not only physicochemical factors (i.e. solubility, permeability and dissolution) but also physiological factors (i.e. intestinal absorption, efflux and first-pass metabolism) may play a crucial role in the process. Therefore, intestinal absorption and secretion are of special importance and should be investigated to fully understand the mechanism responsible for the poor oral absorption of RDS.

To evaluate the intestinal absorption and efflux mechanisms of the compound, this study was designed to investigate the transport characteristics of RDS using Caco-2 cell monolayers as a model of human intestinal epithelium.

Materials and Method

Materials

Rutin deca (H-) sulfonate sodium (purity > 98.7%) was kindly provided by Dr Hu Yongzhou (Department of Medicinal Chemistry, Zhejiang University, P. R. China). Tetrabutyl ammonium bromide was purchased from Wulian Chemical Company (Shanghai, P. R. China). Lucifer yellow, ciclosporin, verapamil and rhodamine-123 were purchased from Sigma (St Louis, MO, USA). All solvents used were of HPLC grade and all chemicals were of analytical grade.

Cell culture

Caco-2 cells obtained from the Chinese Academy of Medical Sciences (CAMS, Beijing, P. R. China) were cultured in high glucose Dulbecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (Gibco), 1% non-essential amino acid (Gibco) and 100 U mL^{-1} antibiotic–antimycotic solution. Cells were grown in a humidified atmosphere of 5%CO_2 at 37°C.

After reaching 80% confluency, Caco-2 cells were harvested with 0.25% trypsin–EDTA solution and seeded in Transwell inserts (12 mm i.d.; catalogue no. 3460; Corning Coster Corp.) in 12-well plates at a density of 1.0×10^5 cells/cm^2. Culture medium was replaced every other day for the first 14 days and daily thereafter for next 7 days until the monolayers expressed differentiated properties that closely resemble morphologic and functional characteristics of normal enterocytes.

Transport experiments

Caco-2 cells in Transwells at passage 90 ~ 106 were used for transport experiment. The integrity of the monolayer was checked by measuring trans-epithelial electrical resistance (TEER) value across the monolayer using a Millicell-ERS voltohmmeter (Millipore) and monitoring the permeability of the paracellular leakage marker Lucifer yellow across the monolayer (Walgren et al 1998). The cell monolayers were considered tight enough for transport experiments when the apparent permeability (P_{app}) for Lucifer yellow was $< 0.2 \times 10^{-6}$ cm s^{-1} and the TEER value was $> 550\,\Omega\,cm^2$.

All transport studies were conducted at 37°C unless specified otherwise. Before the experiment, the inserts were washed twice and pre-incubated for 30 min with warm transport medium, Hank's Balanced Salt Solution containing 25 mM of HEPES, pH 7.4. RDS was dissolved in transport buffer to the desired final concentration in the range 50.0–2000 μM. The solutions were sterile filtered and added on either the apical (AP, 0.5 mL) or basolateral (BL, 1.5 mL) side of the inserts, while the receiving compartment contained the corresponding volume of transport medium. Transport studies were conducted in the absorptive direction (AP→BL) and the efflux direction (BL→AP), separately. After 4 h incubation, 0.5 mL samples were collected from the receiving sides of the cell monolayers for HPLC analysis.

Inhibition studies

The inhibition of efflux of RDS across Caco-2 cell monolayers was investigated in the presence of inhibitors such as ciclosporin, verapamil and rhodamine-123. To determine the kinetics of inhibition, the absorptive and efflux P_{app} of the compound were determined at various substrate concentrations (100.0 ~ 1000 μM) and fixed inhibitor concentration. A Lineweaver–Burk plot was constructed with the reciprocal of transport rate (1/V) versus the reciprocal of substrate concentration (1/C) to determine the type of inhibition (Sha & Fang 2004).

The inhibition degree of RDS by the inhibitors was calculated according to equation 1.

$$\text{Inhibition degree} = [1 - (iP_{app\ BL\text{-}AP} - iP_{app\ AP\text{-}BL})/(P_{app\ BL\text{-}AP} - P_{app\ AP\text{-}BL})] \times 100\% \quad (1)$$

where $P_{app\ BL\text{-}AP}$ and $P_{app\ AP\text{-}BL}$ are the BL→AP and AP→BL permeability of RDS alone and $iP_{app\ BL\text{-}AP}$ and $iP_{app\ AP\text{-}BL}$ are the BL→AP and AP→BL permeability of RDS in the presence of certain inhibitors (Balimane et al 2004).

Ion-pairing reversed-phase high-performance liquid chromatography (RP-HPLC) analysis of RDS

The concentration of RDS was analysed by ion-pairing RP-HPLC after centrifugation of the samples at 10 000 rev min^{-1} for 8 min. Tetrabutyl ammonium bromide (TBA) was used as ion-pairing reagent (Wang et al 2004). The HPLC conditions were as follows: Shimadzu LC-2010C HPLC system with UV detection at 254 nm, an Aglient Zorbax Eclipse XDB-C8 column (5 μm, 150 × 4.6 mm i.d.) with an ODS guard column (10 μm, 10 × 5 mm i.d.). The mobile phase consisted of acetonitrile–0.01 M phosphate buffer solution containing 25 mM TBA (pH 7.4) (50:50, v/v) at a flow rate of 1.0 mL min^{-1}. The injection volume was 80 μL.

The results demonstrated that this method was specific for determining RDS. Calibration curves were constructed by performing a regression linear analysis of the

peak area versus the concentration. The calibration curves of RDS were linear over the concentration range 0.03–3.00 μM (i.e. Y = 59506X–395.64, r = 0.9998). The intra- and inter-day precision and accuracy were analysed at concentrations of 0.03, 0.30 and 3.00 μM in five replicates within one day and on five consecutive days, respectively. The intra-day coefficients of variation were 5.5%, 2.8% and 0.5%, respectively. For the same concentration range, the inter-day coefficients of variation were 8.5%, 4.0% and 1.5%, respectively. The method recoveries at concentrations of 0.03, 0.30 and 3.00 μM were 98.2%, 101.3% and 99.4%, respectively. The limit of detection (lowest concentration that can be detected; signal-to-noise ratio 3) was 0.01 μM. The limit of quantification (lowest concentration that can be quantitatively determined with suitable precision and accuracy; signal-to-noise ratio >10) for RDS was 0.03 μM (RSD $<8.5\%$, n = 5).

Calculations and statistics

Rates of transport (V) of RDS were obtained by equation 2. Permeability of RDS was estimated by calculating P_{app} according to equation 3. The polarized transport was measured by efflux ratio shown as equation 4.

$$V = dQ/(dt \cdot A) \quad (2)$$

$$P_{app} = dQ/(dt \cdot A \cdot Co) \quad (3)$$

$$\text{Efflux ratio} = P_{app\ (BL\text{-}AP)}/P_{app\ (AP\text{-}BL)} \quad (4)$$

where Co is the initial concentration in the donor compartment and A is the surface area of the monolayer. dQ/dt is the rate of appearance of RDS on the receiver side. $P_{app\ BL\text{-}AP}$ and $P_{app\ AP\text{-}BL}$ are the BL→AP and AP→BL permeability of RDS.

Results are given as mean ± s.d. Nonparametric data were analysed by the Kruskal–Wallis test followed by the Dunn's test, or by the Mann–Whitney *U*-test. $P < 0.05$ was considered to be statistically significant.

Results

Absorptive and secretory transport of RDS across Caco-2 cell monolayers

Transport of RDS through Caco-2 cell monolayers occurred in both AP→BL and BL→AP directions. The secretory efflux transport of RDS was 1.4 ~ 4.5-fold greater than the corresponding absorptive transport at concentrations of 50.0 ~ 2000 μM.

In the absorptive direction, the rate of transepithelial transport of RDS increased with an increase in concentration. An Eadie–Hofstee plot of data showed that the AP→BL transport was mediated both by saturable and non-saturable processes and more than one active carrier participated in the transport process (Weierstall et al 1999; Haritos et al 2000; Yamaguchi et al 2001). In the concentration range studied, the secretory transport was not saturated. The efflux P_{app} increased from 6.16 to 11.21×10^{-8} cm s^{-1} as the concentration increased from 50.0 to 250.0 μM and stabilized thereafter (Figure 1).

Effect of pH on the transepithelial transport of RDS

The transepithelial transport of RDS by Caco-2 cell monolayers was examined at pH 6.0, 6.5, 7.0 and 7.4 on the AP side, and at pH 7.4 on the BL side constantly. The transepithelial transport of RDS was pH dependent and its permeability was slightly enhanced at weakly acidic pH on the AP side (Figure 2).

Effect of temperature on the transepithelial transport of RDS

Bi-directional transport of RDS across Caco-2 cell monolayers was investigated at both 37 and 4°C. The permeability of both AP→BL and BL→AP transport of RDS at 4°C was significantly lower than at 37°C ($P < 0.05$, Mann–Whitney *U*-test). The polarized transport that

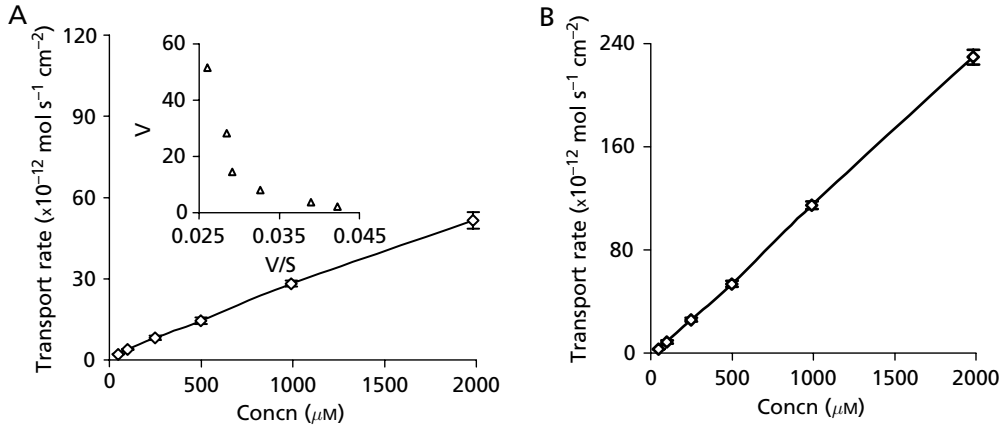

Figure 1 Transport rate of RDS across Caco-2 cell monolayers in the absorptive (A) and secretory (B) direction. The inset represents the Eadie–Hofstee transformation of the data for the absorptive transport. Each data point represents the mean ± s.d. for three independent monolayers.

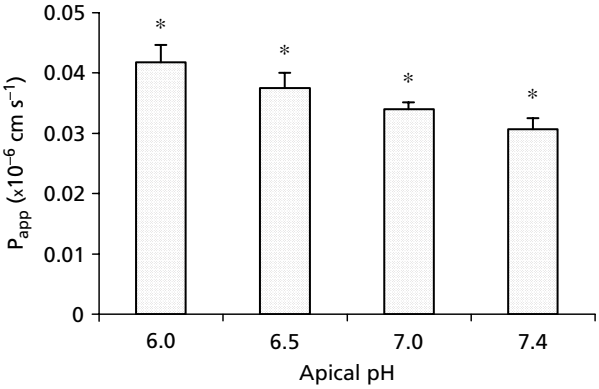

Figure 2 Effect of pH on the transepithelial transport of RDS across Caco-2 cell monolayers. Caco-2 cell monolayers were incubated with RDS (250.0 μM) at 37°C, added to the apical side. The pH of the apical side was 6.0, 6.5, 7.0 or 7.4, and the pH of the basolateral side was 7.4. Each value represents the mean ± s.d. for three independent monolayers. *$P < 0.05$, compared with values at pH 7.4 (Kruskal–Wallis test followed by Dunn's test).

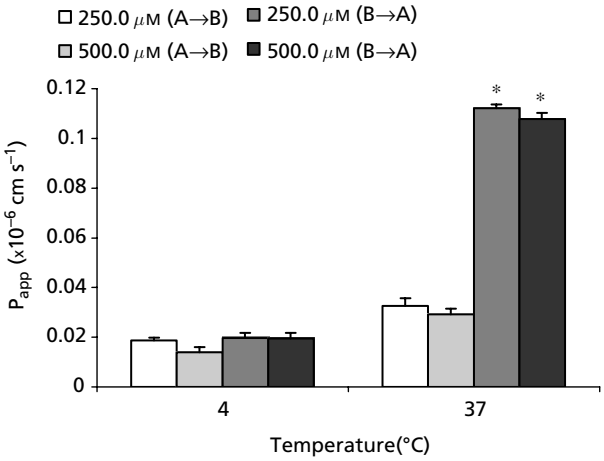

Figure 3 Effect of temperature on the transport of RDS across Caco-2 cell monolayers. The experiments were performed at different temperatures with the compound concentrations of 250.0 and 500.0 μM. Each value represents the mean ± s.d. for three independent monolayers. P_{app} at 4°C was significantly lower than that at 37°C ($P < 0.05$, Mann–Whitney U-test). The difference between the P_{app} of apical-to-basolateral direction (A→B) and basolateral-to-apical direction (B→A) was statistically significant at two concentrations at 37°C (*$P < 0.05$, Kruskal–Wallis test followed by Dunn's test).

could be obviously observed at 37°C almost disappeared when the temperature dropped to 4°C (Figure 3).

Effect of potential inhibitors on the transepithelial transport of RDS

The bi-directional transport of RDS suggested that it was likely to be mediated by active transporters. Therefore, the inhibition of efflux of RDS across Caco-2 cell monolayers was investigated in the presence of two typical P-gp/MRP2 inhibitors, ciclosporin (10 μM) and verapamil (100 μM).

Ciclosporin increased the rate of apical-to-basolateral transport but decreased the rate of basolateral-to-apical transport. When verapamil was used, the transport rate in the secretory direction appeared to decrease slightly while the absorptive transport showed distinct enhancement, which was different to the pattern shown by ciclosporin (Figure 4, Table 1).

It was found from the Lineweaver–Burk plot that ciclosporin competitively inhibited the efflux of RDS and verapamil acted as an uncompetitive inhibitor (Figure 4).

The interaction between RDS and rhodamine-123 during the transport process

The interaction was examined by determining the bi-directional transport of RDS in the presence of rhodamine-123 (5.0 μM) and by determining the bi-directional transport of rhodamine-123 in the presence of RDS (500.0 μM).

The results demonstrated a significant reduction of RDS transport from the BL to AP side with the presence of rhodamine-123 in comparison with control experiments. Meanwhile, the efflux ratio of RDS was reduced to 1 by the competitive inhibition of rhodamine-123 in the transport process (Table 1, Figure 4). In the control experiment, the efflux permeability of rhodamine-123 was about 80-fold higher than the corresponding absorptive permeability. RDS at a concentration of 500.0 μM brought the efflux ratio close to 8 (Figure 5).

Discussion

A preliminary experiment showed that RDS was stable in the transport medium at 37°C. During the incubation period, quercetin was neither detected in the transport medium nor within Caco-2 cells, which implied that this compound was not hydrolysed to quercetin. The integrity of the Caco-2 cell monolayers was monitored during the transport experiment. At the end of the 4-h incubation period, the TEER values of all monolayers remained as much as 90% of the original and the permeability of Lucifer yellow was still $< 0.2 \times 10^{-6}$ cm s^{-1}.

The transport of RDS was shown to be concentration, temperature and pH dependent. The pH may influence the structure of the tight junctions of Caco-2 cell monolayers, which provide a selective barrier for drug permeability. Furthermore, pH change may also result in a change in the activity of the cells with respect to several carrier-mediated transport systems. Temperature changes may affect the diffusivity of drug, membrane fluidity, transporter activity, intracellular trafficking and other factors that may contribute to change in permeability. It is important to note that reduced permeability at lower temperature can also be attributed to inhibition of the carrier-mediated transport mechanism. Therefore, it is assumed that lower

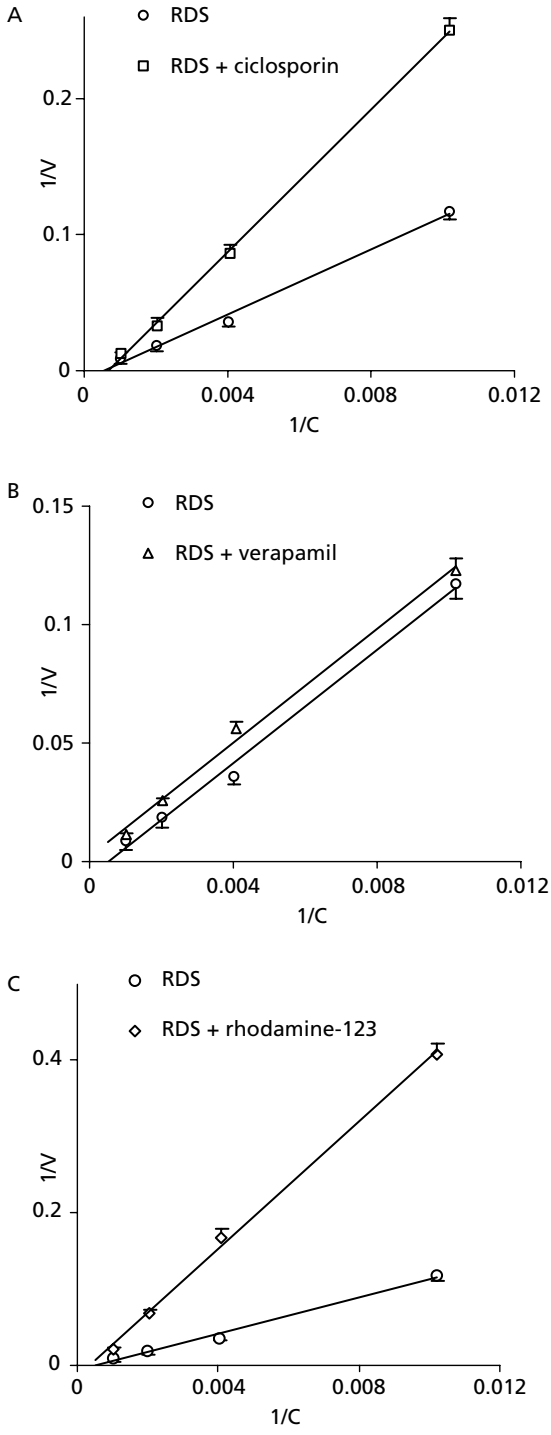

Figure 4 Lineweaver–Burk plot of inhibition of RDS efflux across Caco-2 cell monolayers in the presence of ciclosporin (A), verapamil (B) or rhodamine-123 (C). Each value represents the mean ± s.d. for three independent monolayers. $P < 0.05$, comparison of slope of the plots in A and C (Mann–Whitney *U*-test).

temperatures will reduce the contribution of energy-dependent mechanisms, such as the P-gp efflux system, to the drug transport process (El-Sayed et al 2003).

The polarized transport was due to the presence of efflux carriers at the apical surface that favours secretory transport and inhibits absorptive transport. In the absorptive transport, the curvilinear Eadie–Hofstee graph suggested more than one carrier was involved. The transport might occur in the presence of high-affinity, low-capacity and low-affinity, high-capacity carrier systems (Leonardi et al 1998). The polarized efflux of RDS was competitively inhibited by ciclosporin, a well-known inhibitor of P-gp and MRP2, suggesting the involvement of multiple efflux transporters, including P-gp, in the efflux of RDS. Further confirmation of this hypothesis was obtained by performing inhibition studies with verapamil, which is also a potent inhibitor of P-gp. Rhodamine-123 is selectively transported in the Caco-2 cell line by P-gp and has often been used to study the functionality of P-gp. In this study, rhodamine-123 was used as a substrate to study the effect of RDS on P-gp activity. The addition of RDS significantly lowered the efflux ratio of rhodamine-123 in a fashion indicative of P-gp activity suppression (Phung-Ba et al 1995). Previous studies have demonstrated that P-gp is vulnerable to inhibition, activation or induction by herbal constituents. Many herbal constituents, in particular flavonoids, were reported to modulate P-gp by directly interacting with the vicinal ATP-binding site, the steroid-binding site or the substrate-binding site (Maki et al 2003; Zhou et al 2004). The modulation of P-gp activity and expression may result in altered absorption and bioavailability of drugs that are P-gp substrates (Patel et al 2004). Furthermore, many modulators of P-gp have the potential to modulate cytochrome P450 activity and thus participate in drug-metabolizing enzyme systems (Eagling et al 1999; Durr et al 2000; Ioannides 2002). It is highly possible that cytochrome P450 and P-gp present a barrier to reduce the oral bioavailability of RDS synergistically.

Altogether, these results showed that RDS behaved as an inhibitor of P-gp function and as a substrate of the transporter. It is clear that P-gp plays a pivotal role in the pharmacokinetics of a wide array of compounds by impacting them at various stages of their absorption, distribution and elimination (Suzuki & Sugiyama 2000). The screening of discovery compounds for their potential to interact with P-gp, either as a substrate or as an inhibitor, is becoming very critical. Previous studies indicated that P-gp recognized substrates by recognition elements formed by two or three electron donor groups with a fixed spatial separation and the general feature of P-gp substrates was their relatively hydrophobic, amphiphilic nature (Sandt et al 2000). However, RDS is a hydrophilic macromolecular compound, which is quite different from the general features.

In this study, these inhibitors of P-gp inhibited the efflux of RDS in different manners. The Lineweaver–Burk plots in Figure 4 visualized the different patterns of effect of these inhibitors. One interpretation for the phenomena is that P-gp possesses two or even three substrate binding sites with different substrate affinities (Greenberger et al 1990; Ekins et al 1998; Korzekwa et al 1998; Houston & Kenworthy 2000; Crowe 2002). He & Liu (2002) studied the mechanism of the ATP-dependent

Table 1 Effect of inhibitors on transport of RDS across Caco-2 cell monolayers

Concn (μM)	Efflux ratio				Inhibition degree (%)		
	Control	Ciclosporin	Verapamil	Rho-123	Ciclosporin	Verapamil	Rho-123
100.0	2.23	0.85	0.28	1.03	115.2	589.5	98.7
250.0	3.43	1.04	0.33	0.94	97.7	284.9	102.1
500.0	3.70	1.44	0.30	0.93	76.2	174.3	102.7
1000	4.07	2.09	0.22	0.96	51.3	100.6	102.4

Caco-2 cell monolayers were incubated at 37°C for 4 h with the compound added either to the apical or basolateral side of the cell monolayers. Ciclosporin (10 μM), verapamil (100 μM) or rhodamine-123 (5 μM) was added to both the apical and basolateral side.

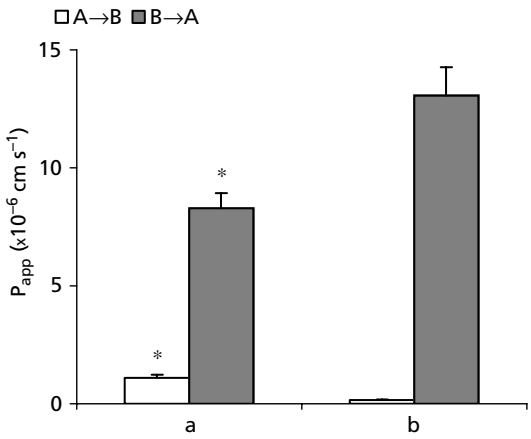

Figure 5 The permeability of rhodamine-123 across Caco-2 cell monolayers with (a) and without (b) the presence of RDS (500.0 μM). A→B represents the apical-to-basolateral transport and B→A represents transport in the basolateral-to-apical direction. Each value represents the mean ± s.d. for three independent monolayers. *$P < 0.05$, P_{app} of rhodamine-123 in the presence of RDS compared with that without the compound (Mann–Whitney U-test).

interaction of P-gp with various multidrug resistance (MDR) reversal agents. Their findings showed that ciclosporin and verapamil could bind P-gp, either on overlapping sites or distant but interacting sites, and thus interacted with P-gp and altered its ATPase activity in different manners. Other researchers also demonstrated cooperative allosteric interactions between ciclosporin and ATP, and antico-operative allosteric interactions between verapamil and ATP in immobilized P-gp (Lu et al 2001; Ekins et al 2002). Therefore, the presence of multiple drug binding sites may account for the different effects of the two inhibitors on the transport of RDS.

Furthermore, an additional carrier mechanism may be involved in the polarized transport of RDS because the absorptive permeability decreased as the concentration increased, even though secretory permeability remained the same. If P-gp were the only transporter involved in the transport of RDS, then we would not expect to produce the efflux ratio that increased as the concentration increased. The assumption is further supported by the fact that an increase in the concentration of RDS, while increasing the rate of transport, did not lead to a proportional increase in the transport rates (Hu & Chen 2004). Moreover, as the inhibition of RDS transport by ciclosporin and verapamil was not complete, it was possible that several efflux transporters worked in co-ordination as a protective mechanism to interpret the poor bioavailability of the compound. We studied the effect of MRP inhibitors on the transport of RDS. Indometacin and probenecid, two non-specific inhibitors of MRPs, enhanced the absorptive transport and reduced the secretory efflux, which suggested that MRP might participate in the transepithelial transport of the compound.

Conclusions

This study suggested that several efflux transporters were capable of mediating the absorption and efflux of RDS, and that they might play a significant role in limiting the oral absorption of this hydrophilic macromolecular compound. RDS was likely to be a substrate of P-gp. Its interaction with P-gp might have important consequences for the pharmacokinetics and toxicity of this compound. Since the evidence suggested the involvement of multiple carriers in the transport of RDS across intestinal epithelial barriers, additional studies are needed to further clarify the precise mechanisms of transport of this hydrophilic macromolecular compound.

References

Balimane, P. V., Patel, K., Marino, A., Chong, S. (2004) Utility of 96 well Caco-2 cell system for increased throughput of P-gp screening in drug discovery. *Eur. J. Pharm. Biopharm.* **58**: 99–105

Crowe, A. (2002) The influence of P-glycoprotein on morphine transport in Caco-2 cells. Comparison with paclitaxel. *Eur. J. Pharmcol.* **440**: 7–16

Durr, D., Stieger, B., Kullak-Ublick, G. A., Rentsch, K. M., Steinert, H. C., Meier, P. J., Fattinger, K. (2000) St John's Wort induces intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4. *Clin. Pharmacol. Ther.* **68**: 598–604

Eagling, V. A., Profit, L., Back, D. J. (1999) Inhibition of the CYP3A4-mediated metabolism and P-glycoprotein-mediated transport of the HIV-1 protease inhibitor saquinavir by

grapefruit juice components. *Br. J. Clin. Pharmacol.* **48**: 543–552

Ekins, S., Ring, B. J., Binkley, S. N., Hall, S. D., Wrighton, S. A. (1998) Autoactivation and activation of cytochrome P450s. *Int. J. Clin. Pharmacol. Ther.* **36**: 642–651

Ekins, S., Kim, R. B., Leake, B. F., Dantzig, A. H., Schuetz, E. G., Lan, L. B., Yasuda, K., Shepard, R. L., Winter, M., Schuetz, J. D., Wikel, J. H., Wrighton, S. A. (2002) Application of three-dimensional quantitative structure-activity relationships of P-glycoprotein inhibitors and substrates. *Mol. Pharmacol.* **61**: 974–981

El-Sayed, M., Rhodes, C. A., Ginski, M., Ghandehari, H. (2003) Transport mechanism(s) of poly (amidoamine) dendrimers across Caco-2 cell monolayers. *Int. J. Pharm.* **265**: 151–157

Greenberger, L. M., Yang, C.-P. H., Gindin, E., Horwitz, S. B. (1990) Photoaffinity probes for the α1-adrenergic receptor and the calcium channel bind to a common domain in P-glycoprotein. *J. Biol. Chem.* **265**: 4394–4401

Haritos, V. S., Ghabrial, H., Ahokas, J. T., Ching, M. S. (2000) Role of cytochrome P450 2D6 (CYP2D6) in the stereospecific metabolism of E- and Z-doxepin. *Pharmacogenetics* **10**: 591–603

He, L., Liu, G. Q. (2002) Interaction of multidrug resistance reversal agents with P-glycoprotein ATPase activity on blood-brain barrier. *Acta Pharmacol. Sin.* **23**: 423–429

Hidalgo, I. J., Li, J. (1996) Carrier-mediated transport and efflux mechanisms in Caco-2 cells. *Adv. Drug Deliv. Res.* **22**: 53–66

Hidalgo, I. J., Raub, T. J., Borchardt, R. T. (1989) Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology* **96**: 736–749

Houston, J. B., Kenworthy, K. E. (2000) In vitro-in vivo scaling of CYP kinetic data not consistent with the classical Michaelis-Menten model. *Drug. Metab. Dispos.* **28**: 246–254

Hu, M., Chen, J. (2004) Nucleobase- and p-glycoprotein-mediated transport of AG337 in a Caco-2 culture Model. *Mol. Pharmacol.* **1**: 194–200

Ioannides, C. (2002) Pharmacokinetic interactions between herbal remedies and medicinal drugs. *Xenobiotica* **32**: 451–478

Korzekwa, K. R., Krishnamachary, N., Shou, M., Ogai, A. R. A. P., Rettie, A. E., Gonzalez, F. J. (1998) Evaluation of atypical cytochrome P450 kinetics with two-substrate-models: evidence that multiple substrates can simultaneously bind to cytochrome P450 active sites. *Biochemistry* **37**: 4137–4147

Leonardi, M. G., Casartelli, M., Parenti, P., Giordana. B. (1998) Evidence for a low-affinity, high-capacity uniport for amino acids in *Bombyx mori* larval midgut. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **274**: 1372–1375

Lu, L., Leonessa, F., Clarke, R., Wainer, I. W. (2001) Competitive and allosteric interactions in ligand binding to P-glycoprotein as observed on an immobilized P-glycoprotein liquid chromatographic stationary phase. *Mol. Pharmacol.* **59**: 62–68

Maki, N., Hafkemeyer, P., Dey, S. (2003) Allosteric modulation of human P-glycoprotein. Inhibition of transport by preventing substrate translocation and dissociation. *J. Biol. Chem.* **278**: 18132–18139

Mu, Q. Z., Shen, Y. M., Zhou, Q. L. (1998) China Patent ZL98116970

Nair, V. G., Bernstein, S. (1983) European Patent 0075626

Patel, J., Buddha, B., Dey, S., Pal, D., Mitra, A. K. (2004) In vitro interaction of the HIV protease inhibitor ritonavir with herbal constituents: changes in P-gp and CYP3A4 activity. *Am. J. Ther.* **11**: 262–277

Phung-Ba, V., Warnery, A., Scherman, D., Wils, P. (1995) Interaction of pristinamycin I_A with P-glycoprotein in human intestinal epithelial cells. *Eur. J. Pharmacol.* **288**: 187–192

Sandt, I., Blom-Roosemalen, M., Boer, A., Breimer, D. (2000) Specificity of doxorubicin versus rhodamine-123 in assessing P-glycoprotein functionality in the LLC-PK1, LLC-PK1:MDR1 and Caco-2 cell lines. *Eur. J. Pharm. Sci.* **11**: 207–214

Sha, X., Fang, X. (2004) Transport characteristics of 9-nitro-camptothecin in the human intestinal cell line Caco-2 and everted gut sacs. *Int. J. Pharm.* **272**: 161–171

Suzuki, H., Sugiyama, Y. (2000) Role of metabolic enzymes and efflux transporters in the absorption of drugs from the small intestine. *Eur. J. Pharm. Sci.* **12**: 3–12

Walgren, R. A., Walle, U. K., Walle, T. (1998) Transport of quercetin and its glucosides across human intestinal epithelial Caco-2 cells. *Biochem. Pharmacol.* **55**: 1721–1727

Wang, X. J., Tang, Y. H., Yao, T. W., Zeng, S. (2004) Separation of rutin nona (H-) and deca (H-) sulfonate sodium by ion-pairing reversed-phase liquid chromatography. *J. Chromatogr. A* **1036**: 229–232

Weierstall, T., Hollenberg, C. P., Boles, E. (1999) Cloning and characterization of three genes *(SUT1–3)* encoding glucose transporters of the yeast *Pichia stipitis*. *Mol. Microbiol.* **31**: 871

Yamaguchi, H., Yano, I., Saito H., Inui, K. (2001) Transport characteristics of grepafloxacin and levofloxacin in the human intestinal cell line Caco-2. *Eur. J. Pharmacol.* **431**: 297–303

Zhou, S., Lim, L. Y., Chowbay, B. (2004) Herbal modulation of P-glycoprotein. *Drug Metab. Rev.* **36**: 57–104