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Transport characteristics of zolmitriptan in a human intestinal epithelial cell line Caco-2

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Abstract

The intestinal absorption characteristics and the efflux mechanisms of zolmitriptan, a new generation and highly selective 5-$HT_{1B/1D}$ receptor agonist used in the acute oral treatment of migraine, were investigated. A human intestinal cell line, Caco-2, was used as an in-vitro model of the intestinal mucosa to assess transepithelial transport of zolmitriptan. In the Caco-2 cells, the absorptive transport of zolmitriptan was pH dependent and the transport was enhanced at weakly alkali pH on the apical side. No concentration dependence and saturation were observed for the apical-to-basolateral and basolateral-to-apical transport of zolmitriptan at a concentration of 0.1–10 mM. The permeability ratio value was about 1.5–2.6 at a concentration of 0.1–2.0 mM. Inhibition experiments using verapamil, nifedipine and nimodipine as inhibitors were studied and indicated that P-glycoprotein participated in the transport of zolmitriptan. Inhibition of the Na^+–H^+ exchanger with amiloride resulted in a significant increase in absorption and a slight inhibition in secretion. This suggests that the Na^+–H^+ exchanger may be involved in the transport of zolmitriptan. The results indicated that the transport of zolmitriptan was mediated by both passive diffusion and active transport. A series of drug–drug interaction experiments were carried out between zolmitriptan and some drugs that may be co-administrated with zolmitriptan in the clinic. The results indicated that flunarizine, cetirizine, propranolol and atenolol potently decreased both the apical-to-basolateral and basolateral-to-apical transport rate of zolmitriptan. Cimetidine and aspirin slightly inhibited the apical-to-basolateral transport of zolmitriptan, but significantly decreased the basolateral-to-apical transport of zolmitriptan. Thus, the absorption drug–drug interactions should be considered when these drugs are co-administrated with zolmitriptan in the clinic.

Introduction

Zolmitriptan is a new generation and highly selective 5-$HT_{1B/1D}$ receptor agonist used in the acute oral treatment of migraine and it has recently been used as an effective neuroendocrine probe of 5-HT_{1D} receptor function in humans (Schoenen & Sawyer 1997; Whale et al 1999). Potential drug interactions can take place in the clinic when zolmitriptan is co-administrated with some other drugs. Co-administration of propranolol resulted in a 56% and 37% increase in the C_{max} and AUC of zolmitriptan respectively; mean t½ was prolonged from 3.1 to 4.0 h (Peck et al 1997). Moclobemide, a monoamine oxidase A inhibitor, decreased the clearance of zolmitriptan (Rolan 1997). In a previous study, we found that fluvoxamine and diphenytriazol potently inhibited zolmitriptan *N*-demethylase activity catalysed by CYP1A2 in-vitro, and diazepam and propranolol elicited a slight inhibitory effect on the metabolism of zolmitriptan in-vitro (Yu et al 2003).

It is well known that the intestinal absorption rate is very important to the bioavailability of oral drugs. Many factors can affect the intestinal absorption rate of drugs, such as drug dissolution characteristics, luminal pH, luminal contents and drug transport proteins (Doherty & Charman 2002). Good permeability through intestinal membranes leads to adequate systemic absorption. A cell model system, such as Caco-2 cells, is commonly used in drug discovery and development as a predictive tool to estimate intestinal absorption and drug–drug interactions.

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 family. They belong to the ATP-binding

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cassette 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's absorption potential, studying the transport mechanism of drugs and elucidating the metabolism of drugs.

The clinical implications of drug–drug interactions are significant. Drug–drug interactions inevitably result in increased clearance or reduced absorption of affected drugs. These changes in exposure can alter the safety and efficacy profile of a drug and/or its active metabolites in important ways (Food and Drug Administration 2004). P-gp limits drug absorption by extruding the drug from epithelial cells into the intestinal lumen. As with cytochrome P450 enzymes, inhibition and induction of P-gp has been reported as a cause of drug–drug interactions. Drug interactions may occur when P-gp substrates and inhibitors or inducers are co-administered.

In the clinic, zolmitriptan may be co-administrated with other drugs that may affect its absorption. We previously reported an uptake study of zolmitiptan in Caco-2 cells (Yu et al 2006). Uptake and transport studies are two important ways of exploring a drug's absorption characteristics and so the current work focused on the transport characteristics of zolmitriptan. One of the aims of this study was to investigate the transport characteristics of zolmitriptan using Caco-2 cell monolayers as a model of human intestinal epithelium, the other aim was to study the possible effects of some drugs on the transport of zolmitriptan in-vitro.

Materials and Methods

Chemicals and reagents

Zolmitriptan, sumatriptan succinate and acetylsalicylic acid were provided by the Department of Chemistry, Zhejiang University (Hangzhou, China). Verapamil hydrochloride, propranolol hydrochloride, amiloride hydrochloride, nimodipine, nifedipine, flunarizine, cimetidine, atenolol, cetirizine and lucifer yellow CH were purchased from Sigma (St Louis, MO, USA). All other chemicals were of reagent grade. The stock solutions of zolmitriptan and other compounds were made in dimethylsulfoxide. The concentration of dimethylsulfoxide in the final solutions was 0.5%, a concentration that has been shown not to affect the integrity of Caco-2 monolayers.

The Caco-2 cell line was obtained from the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences Peking Union Medical College. High-glucose Dulbecco's modified Eagle's medium, fetal bovine serum, non-essential amino acids, L-glutamine and antibiotic mixture (10 000 IU mL^{-1} penicillin G, 10 000 $\mu g\,mL^{-1}$ streptomycin) were purchased from Gibco Zmitrogen (Life Technologies, Paisley, Scotland, UK). Twelve-well Transwells were purchased from Corning Costar Corporation (Costar, Cambridge, MA, USA).

Cell culture

Caco-2 cells in Transwells at passage 40–70 were used for the transport experiment. Caco-2 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 1% non-essential amino acids and 5% antibiotic–antimycotic solution at 37°C in culture flasks (Thermo Electron Corporation, Marietta, OH, USA) in a humidified air/5% CO_2 atmosphere. After reaching 80% confluency, Caco-2 cells were harvested with trypsin (0.25%)/EDTA (1 mM) and seeded onto polycarbonate filters (0.3-μm pores, 1.13 cm^2 growth area) inside Transwell cell culture chambers at a density of 1×10^5 cells/filter. The culture medium (0.5 mL in the insert and 1.5 mL in the well) was replaced every 48 h for the first 6 days and every 24 h thereafter, and after 18–21 days in culture the Caco-2 monolayer was utilized for the following experiments.

The integrity of the cell monolayers was evaluated by measuring the transepithelial electrical resistance (TEER) value with a Millicell-ERS voltohmmeter (Millipore Corp., Bedford, MA, USA) and monitoring permeability of the paracellular leakage marker lucifer yellow CH across the monolayer (Walgren et al 1998). The cell monolayers were considered tight enough for transport experiments when the TEER value was >400 $\Omega\,cm^{-2}$ and the apparent permeability (P_{app}) for lucifer yellow CH was $<0.5 \times 10^{-6}$ cm s^{-1}.

Transport studies

All transport studies were conducted at 37°C unless otherwise specified. Before the experiment, the inserts were washed twice with warm Hank's balanced salt solution (HBSS) containing 25 mM HEPES, pH 7.4. After each wash, the plates were returned to the incubator for 30 min. TEER values were measured after the final wash. The buffer was then replaced with fresh HBSS/HEPES buffer on one side of the cell layer and zolmitriptan in HBSS/HEPES buffer on the other side. The apical (AP) side of the cell layer (insert) contained 0.5 mL, and the basolateral (BL) side (well) contained 1.5 mL. In each experiment, three inserts were used for each treatment. When zolmitriptan (0.1–2.0 mM) was added on one side of the cell layer, 100-μL samples were taken from the other side and were replaced with equal volumes of fresh buffer every 0.5 h. The samples were filtrated with 0.45-μm micro membrane. The cumulative transport was calculated after correction for dilution. The concentration of the samples was determined by high-performance liquid chromatography.

Concentration dependence of zolmitriptan transport

The transport of zolmitriptan (0.1–10 mM) from the AP to BL side and BL to AP side of the Caco-2 monolayers was followed for 2 h. To assess the effect of zolmitriptan concentration on the monolayer integrity, the TEER was measured again at the end of the transport experiments and no significant changes were detected.

Inhibition studies

The transport of 0.8 mM zolmitriptan both in the AP-to-BL direction and the BL-to-AP direction was determined after 2 h in the presence of different inhibitors. All of the inhibitors were added to the AP side at a concentration of 0.5 mM.

Determination of zolmitriptan

All the samples described above were analysed by reverse phase high-performance liquid chromatography, using a 5-μm reverse phase column (Shimpack CLC-ODS 150 mm × 6.0 mm; Shimadzu, Japan). The mobile phase was a mixture of acetonitrile/0.01 M KH_2PO_4–K_2HPO_4 (pH 7.5) (25/75, v/v) at a flow rate of 1.0 mL min^{-1}. The detection wavelength was 229 nm and the retention time of zolmitriptan was approximately 7 min.

Data analysis

The P_{app} values were calculated as $V/AC_0 \times dC/dt$ (cm s^{-1}), where V is the volume of the solution in the receiving compartment, A is the membrane surface area, C_0 is the initial concentration in the donor compartment and dC/dt is the change in drug concentration in the receiver solution over time. The permeability ratio (P_{ratio}) was calculated as $P_{app,\ BL\text{-}to\text{-}AP}/P_{app,\ AP\text{-}to\text{-}BL}$, where $P_{app,\ BL\text{-}to\text{-}AP}$ is the permeability from the basolateral to the apical side (cm s^{-1}) and $P_{app,\ AP\text{-}to\text{-}BL}$ is the permeability from the apical to the basolateral side (cm s^{-1}).

All values are presented as a mean ± s.d. Individual differences were evaluated using Dunn's test. Non-parametric data were examined by the Kruskal–Wallis test or by the Mann–Whitney *U*-test. $P < 0.05$ was considered to be statistically significant.

Results

Transepithelial transport of zolmitriptan

The flux of zolmitriptan across Caco-2 cell monolayers when the drug was loaded on either the AP or BL side of the cells is shown in Figure 1. As can be seen, the flux was essentially linear for up to 2.5 h for all zolmitriptan concentrations studied (0.1–2.0 mM). The mean P_{app} values for the BL-to-AP transport (approx. $2.10 \pm 0.39 \times 10^{-6}$ cm s^{-1}) were significantly higher than those for the AP-to-BL transport (approx. $1.15 \pm 0.15 \times 10^{-6}$ cm s^{-1}) at each zolmitriptan concentration ($P < 0.01$). The P_{ratio} value was approximately 1.5–2.6. This suggests that some active transport pathway such as substrate efflux mediated by P-gp may be involved in the absorption of zolmitriptan.

The influence of the concentration of zolmitriptan on its transport by Caco-2 cell monolayers was examined (Figure 2). The range of concentrations used was limited by the lack of zolmitriptan solubility in HBSS/HEPES buffer. Both AP-to-BL flux and BL-to-AP flux showed concentration dependency and were linear with increasing zolmitriptan concentrations (approx. 0.2–10 mM). This result indicated that passive diffusion may also be involved in the absorption of zolmitriptan.

Effect of pH on the transepithelial transport of zolmitriptan

The transepithelial transport of zolmitriptan by Caco-2 cell monolayers was examined at pH 6.0, 7.0 and 7.4 on the AP side. The transepithelial transport of zolmitriptan was pH dependent and its permeability was significantly enhanced at weakly alkaline pH on the AP side (Figure 3).

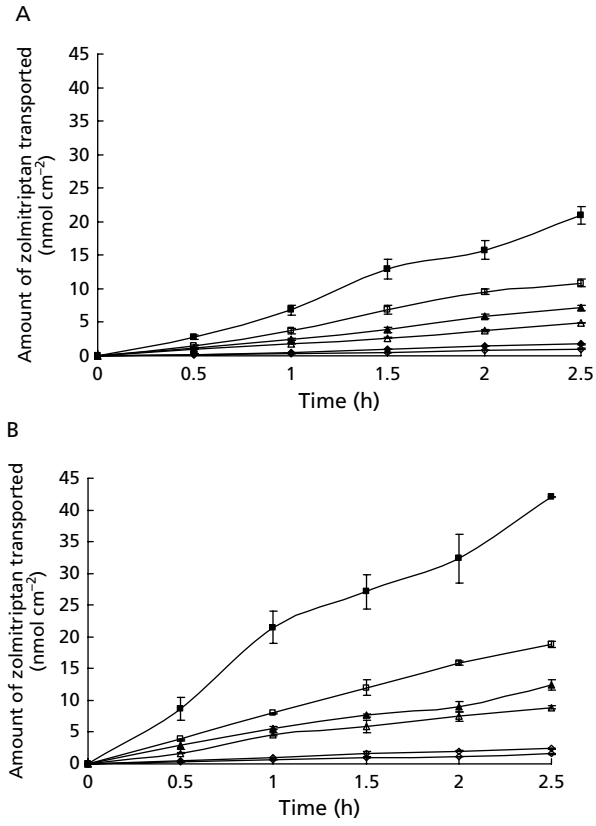

Figure 1 Transepithelial flux of zolmitriptan across the Caco-2 cell monolayer. The zolmitriptan concentrations used were 0.1 mM (◇), 0.2 mM (♦), 0.4 mM (▲), 0.6 mM (Δ), 1.0 mM (□) and 2.0 mM (■). A. Apical-to-basolateral flux; B. basolateral-to-apical flux. Each point is the mean value of three experiments.

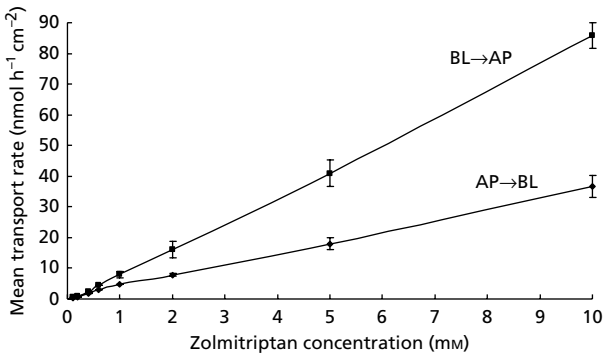

Figure 2 Effect of intracellular concentration on the rate of efflux of zolmitriptan. The intracellular concentrations of zomitriptan are given in Figure 1. AP, apical; BL, basolateral. Each point is the mean value of three experiments.

Effect of P-glycoprotein inhibitors on the transepithelial transport of zolmitriptan

Table 1 shows the effect of typical inhibitors for P-gp on the transepithelial transport of zolmitriptan. Verapamil, nifedipine and nimodipine can all significantly increase the AP-to-BL transport rate of zolmitriptan. At the same time, nifedipine

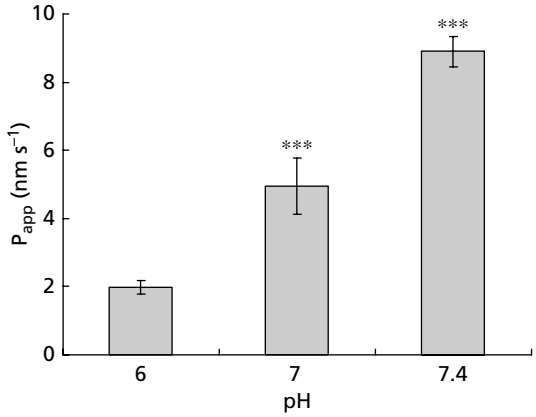

Figure 3 Effect of pH on the transport of zolmitriptan across Caco-2 cell monolayers. Caco-2 cell monolayers were incubated with zolmitriptan (0.8 mmol L^{-1}) at 37°C added to the apical side. The pH of the apical side was 6.0, 7.0 or 7.4, and the pH of the basolateral side was 7.4. Data represent the mean ± s.d. of three different experiments. ***$P < 0.001$, significantly different compared with pH 6.

Table 1 Effect of P-glycoprotein inhibitors on the transport of zolmitriptan in Caco-2 cell monolayers

Compound	Transport rate (nmol cm^{-2}/2 h)	
	Apical-to-basolateral	Basolateral-to-apical
Control	7.94 ± 1.21 (100)	18.09 ± 1.04 (100)
Verapamil	9.86 ± 0.87 (124)*	17.01 ± 0.92 (94)
Nifedipine	12.85 ± 0.87 (162)**	12.52 ± 0.17 (69)**
Nimodipine	17.22 ± 3.20 (217)***	8.19 ± 0.04 (45)***

The transport of 0.8 mM zolmitriptan was determined after 2 h in the absence (control) or presence of 0.5 mM of each of the P-glycoprotein inhibitors, which were added to the apical side. Zolmitriptan was added on either the apical or basolateral side of the monolayers. Transport values are means ± s.d., n = 4. The extent of transport expressed as % of control is given within parentheses. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, significantly different compared with control.

and nimodipine can significantly decrease the BL-to-AP transport. These results indicate that P-gp participates in the transport of zolmitriptan.

Effect of Na^+–H^+ exchanger on transepithelial transport of zolmitriptan

The influence of Na^+–H^+ exchanger on the transport of zolmitriptan on Caco-2 monolayers was studied by the addition of the Na^+–H^+ exchanger inhibitor amiloride (0.5 mM) on the AP side. This concentration has previously been shown to effectively inhibit Na^+–H^+ exchange in Caco-2 cells (Watson et al 1991; Hootman et al 2005). Inhibition of the Na^+–H^+ exchanger with amiloride resulted in a significant increase in zolmitriptan absorption and a slight decrease in zolmitriptan secretion (Figure 4).

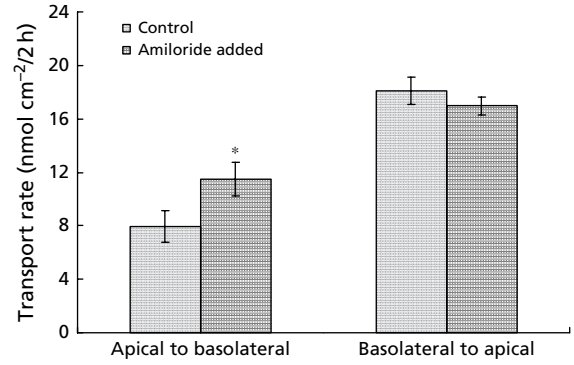

Figure 4 Effect of amiloride on the transport of zolmitriptan across Caco-2 cell monolayers. The transport of 0.8 mM zolmitriptan was determined after 2 h in the absence and presence of 0.5 mM amiloride on apical side. Data represents the mean ± s.d. of three different experiments. *$P < 0.05$, significantly different compared with control.

Effect of different interaction drugs on transepithelial transport of zolmitriptan

A series of experiments using inhibitors were carried out to determine the nature of zolmitriptan transport on both the AP-to-BL and BL-to-AP sides. The results shown in Table 2 indicate that flunarizine, cetirizine, propranolol and atenolol potently decreased both the AP-to-BL and BL-to-AP transport rate of zolmitriptan. Cimetidine slightly inhibited the AP-to-BL transport of zolmitriptan, but significantly decreased the BL-to-AP transport of zolmitriptan. Acetylsalicylic acid did not have a significant effect on the absorption of zolmitriptan. Among these drugs only sumatriptan significantly increased the AP-to-BL transport of zolmitriptan.

Table 2 Effect of some drugs on transport of zolmitriptan in Caco-2 cell monolayers

Compound	Transport rate (nmol cm^{-2}/2 h)	
	Apical-to-basolateral	Basolateral-to-apical
Control	7.94 ± 1.21 (100)	18.09 ± 1.04 (100)
Flunarizine	6.11 ± 0.21 (77)*	7.94 ± 0.46 (44)***
Cetirizine	5.16 ± 0.46 (66)**	11.31 ± 1.04 (64)**
Sumatriptan	11.98 ± 0.96 (151)*	16.39 ± 0.79 (91)
Propranolol	6.74 ± 0.21 (85)*	14.31 ± 0.08 (79)*
Atenolol	6.24 ± 0.54 (78)*	11.77 ± 0.50 (66)**
Cimetidine	7.65 ± 0.67 (96)	14.68 ± 1.04 (81)*
Acetylsalicylic acid	7.57 ± 0.04 (95)	15.85 ± 1.50 (88)

The transport of 0.8 mM zolmitriptan was determined after 2 h in the absence (control) or presence of 0.5 mM of each of drugs which was added to the apical side. Zolmitriptan was added on either the apical or basolateral side of the monolayers. Transport values are means ± s.d., n = 4. The extent of transport expressed as % of control is given within parentheses. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, significantly different compared with control.

Discussion

The results of this study showed that the AP-to-BL P_{app} was approximately 1.15×10^{-6} cm s^{-1} for zolmitriptan, which was similar to that of sumatriptan (0.93 cm s^{-1}) (Polli et al 2001). According to a previous study (Artursson & Karlsson 1991), a P_{app} value in Caco-2 cells of $>1 \times 10^{-6}$ cm s^{-1} should, in general, be associated with efficient intestinal absorption in humans. Mean absolute bioavailability of zolmitriptan is approximately 40% (Seaber et al 1996), so it is hypothesized that efficient intestinal absorption may be one of the contributory factors that lead to the good oral bioavailability of zolmitriptan.

The transport of drugs across the intestinal epithelium may occur by one or more of four different routes: the passive transcellular and paracellular routes, the carrier mediated route and by transcytosis. Caco-2 monolayers have been used to study drug transport by all four routes. Often, transport is mediated partly by the carrier and partly by passive routes. Since carrier-mediated transport is saturable, the contribution of the passive route will increase with increasing dose. If the drug has a low passive permeability, saturation of the carrier will result in a decreased absorbed fraction. There are also active transporters such as P-gp, which mediate drug transport in the serosal to mucosal direction. In this case, saturation of the carrier could result in an increase in the absorbed fraction of the drug. In this study, both AP-to-BL and BL-to-AP flux showed concentration dependency, and they were linear with increasing zolmitriptan concentrations (approx. 0.2–10 mM). This was similar in an uptake study of zolmitriptan but the uptake rates of zolmitriptan show concave concentration dependency at high concentrations (Yu et al 2006). The results indicate that passive diffusion may be one of the absorption routes of zolmitriptan. At the same time, some active transporters may be involved, such as P-gp.

P-gp efflux resulted in higher BL-to-AP transport than AP-to-BL transport. This assay, where the P_{ratio} was compared with a value of 1, was regarded as the standard for identifying P-gp substrates. Involvement of a P-gp-mediated efflux mechanism was indicated if the P_{ratio} was >2. For a compound with a P_{ratio} of 1.5–2.0, a follow-up experiment with P-gp inhibitors should be performed to confirm if the compound is a P-gp substrate (Polli et al 2001). In the present study, the P_{ratio} value of zolmitriptan was approximately 1.5–2.6. Thus, experiments using verapamil (Karlsson et al 1993; Walle & Walle 1998), nifedipine (Kim et al 1999) and nimodipine (Liu et al 2002; Zhang et al 2003) as inhibitors were carried out which indicated that P-gp participated in the transport of zolmitriptan (Table 1). In our previous study, we found that verapamil, nifedipine and nimodipine significantly increased the uptake of zolmitriptan on the AP side (Yu et al 2006). The uptake and transport results indicated that the transport of zolmitriptan was mediated both by passive diffusion and active transport.

When the proton concentration was decreased at the AP side of the cell monolayers (pH from 6 to 7.4), the AP-to-BL transport rate of zolmitriptan increased by 4 times, indicating that the transport of zolmitriptan was proton gradient dependent (Figure 3). The present results showed that the passive transcellular route was one of the main routes for the absorption of zolmitriptan. As an alkalescence drug, the free zolmitriptan was increased with an increase of pH. This may be one of the reasons leading to a greater transport rate. Inhibition of the Na^+–H^+ exchanger with amiloride (Watson et al 1991; Hootman et al 2005) resulted in a significant increase in absorption and slight inhibition in secretion of zolmitriptan, while inhibition of an organic cation–H^+ exchanger with cimetidine resulted in a significant secretion of zolmitriptan. Additionally, the zolmitriptan uptake was also increased by 25% (AP-to-BL) and 17% (BL-to-AP) with amiloride (Yu et al 2006). The results indicated that the H^+ exchanger can affect the absorption of zolmitrptan, but the mechanism was not clear.

Zolmitriptan is used in the acute oral treatment of migraine, and may be co-administrated with other drugs in the clinic, such as calcium-channel blockers, drugs for psychiatric disorders, non-steroidal anti-inflammatory drugs and β-receptor inhibitors. In an attempt to explain the in-vivo interactions, we investigated the possible in-vitro transport interactions between zomitriptan and some drugs at the cell level. The calcium-channel blockers verapamil, nifedipine and nimodipine can significantly increase the AP-to-BL transport rate. Flunarizine and cetirizine are effective P-gp reversal agents (Barancik et al 1994; He & Liu 2002; Chen et al 2003; Polli et al 2003) and so should inhibit the AP-to-BL transport rate of zolmitriptan. However, flunarizine and cetirizine potently inhibited both AP-to-BL and BL-to-AP transport rate of zolmitriptan. Flunarizine increased the accumulation of zolmitriptan in our previous uptake study (Yu et al 2006). The human intestinal cell line Caco-2 constitutively expresses drug transporters such as peptide transporter, P-gp, organic cation transporter and organic anion transporter (Tsuji & Takanaga 1994), and these may inhibit other drug transporters leading to a decrease of zolmitriptan transport. The results suggested that the pharmacokinetics of zolmitriptan might be changed when zolmitriptan is co-administrated with these drugs in the clinic. Sumatriptan, a P-gp substrate (Evans et al 2003), also significantly increased the AP-to-BL transport and uptake (Yu et al 2006) of zolmitriptan. Peck et al (1997) reported that propranolol increased mean zolmitriptan C_{max} and AUC by 56% and 37% in humans, respectively. Propranolol can inhibit both the AP-to-BL and BL-to-AP transport rate of zolmitriptan, but the efflux inhibition was more difficult than the absorption. In addition, in our previous study we found that propranolol can inhibit the in-vitro metabolism of zolmitriptan (Yu et al 2003). This may be the reason for the increase of C_{max} and AUC of zolmitriptan when zolmitriptan is co-administered with propranolol in the clinic.

Conclusion

The results indicated that the transport of zolmitriptan is mediated by both passive diffusion and active transporters such as P-gp. The efflux of zolmitriptan by P-gp might play a role in limiting the bioavailability of zolmitriptan. Furthermore, the results suggest that co-administration of zolmitriptan with some drugs, such as verapamil, nifedipine, nimodipine and sumatriptan, can increase its absorption in Caco-2 cell

monolayers. Flunarizine, cetirizine, propranolol and atenolol inhibited zolmitriptan AP-to-BL and BL-to-AP transport rates and additional studies are needed to clarify the precise mechanisms.

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