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Zolmitripan uptake by human intestinal epithelial Caco-2 cells

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The oral uptake of zolmitriptan, a novel and highly selectively 5-HT $_{1B/1D}$ receptor agonist, was evaluated in the human epithelial cell line caco-2 that possesses intestinal enterocyte-like properties when cultured *in vitro*. The study demonstrated that zolmitriptan uptake significantly depended upon the extracelluar temperature and pH in the Caco-2 cell. The zolmitriptan uptake at 39 °C was 2.1 fold as that at 23 °C and the zolmitriptan uptake at pH 8.0 was 2.7 fold as that at pH 6.0. The uptake rates of zolmitriptan on both sides increased with increasing zolmitriptan concentration from 0.1 to 10 mmol · L^{-1}, and it shows concave concentration-dependency at high concentration. The uptake rates of zolmitriptan on the basolateral side (BL) were 3–7 times higher than that on the apical side (AP). Verapamil, nimodipine, nifedipine, flunarizine, amiloride and sumatriptan significantly increased the uptake rates of zolmitriptan on the apical sides. Propafenone significantly inhibited the uptake rate of zolmitriptan on both sides. Propranolol and aspirin have no significant effect. The results indicated that the zolmitriptan uptake in Caco-2 cells was temperature, pH and concentration dependent, and was partially counteracted by the action of an outwardly directed efflux pump, presumably p-glycoprotein. Absorption interactions should be considered when P-gp substrates or inhibitors, Na^+-H^+ exchange inhibitors, P-gp ATPase agonists or inhibitors are co-administered with zomitriptan in clinical practice.

1. Introduction

Zolmitriptan ((*S*)-4-[[3-[-(dimethylamine)ethyl]-1H-indol-5-yl]methyl]-2-oxazolidinone) is a novel and highly selective 5-HT 1B/1D receptor agonist used in the acute oral treatment of migraine and has recently been used as an effective neuroendocrine probe of 5-HT1D receptor function in humans (Schoenen and Sawyer 1997; Whale and Bhagwagar 1999). After oral administration to healthy male volunteers, zolmitriptan is rapidly absorbed with 50 ~ 85% of the eventual peak plasma concentration being attained within 1 h after dosing (Seaber et al. 1996) and has dose-proportional pharmacokinetics that is not affected by food to any clinically relevant extent (Emma and Richard 1998).

P-Glycoprotein (P-gp) does limit drug absorption by extruding the drug from epithelial cells into the intestinal lumen. Like cytochrome P450 enzymes, inhibition and induction of P-gp have been reported as causes of drug-drug interactions. Drug interactions may occur when P-gp substrates and inhibitors or inducers are co-administered. The human P-gp is localized on the apical surface of epithelial cells in intestine (Jiunn 2003). The Caco-2 cell line was originally isolated from a human colon adenocarcinoma by Fogh et al. (1977), and is widely used as an *in vitro* model of the human intestinal mucosa (Hidalgo et al. 1989). Caco-2 cells can also be used to characterize the absorption of oral drugs, because the cells exhibit both morphological and functional similarities to the human small intestinal epithelial cells such as cytochrome P450 enzymes and P-glycoprotein (Hilgers et al. 1990).

Zolmitriptan is likely to be co-administered with other drugs in clinic which may affect the absorption of zolmitriptan, such as propranolol (Peck et al. 1997), flunarizine (Lewis et al. 2004), sumatriptan (Lewis et al. 2004), and aspirin (Katsarava and Limmroth 2004). Thus, the aim of this work was to investigate the mechanism of zolmitriptan uptake at the cell level, and to study the possible effect of some drugs to the absorption of zolmitriptan.

2. Investigations and results

2.1. Effect of pH and temperature on the uptake of zolmitriptan

Zolmitriptan uptake on Caco-2 cell monolayer apical sides was pH and temperature dependent. The uptake rate was both found to increase with an increase in pH and temperature (Figs. 1 and 2). The zolmitriptan uptake at pH 8.0 was 2.7 fold as that at pH 6.0, and the zolmitriptan uptake rate at 39 °C was 2.1 fold higher than that at 23 °C.

2.2. Concentration-dependency uptake of zolmitriptan

Zolmitriptan uptake in Caco-2 monolayers, when the drug was loaded on either the apical or basolateral side of the cells at the concentration from 1 to 10 mmol · L^{-1}, is shown in Fig. 3. The uptake rates of zolmitriptan on both sides increased with the zolmitriptan concentration from increasing 1 to 10 mmol · L^{-1}, and it shows concave concentration-dependency at high concentration. The uptake

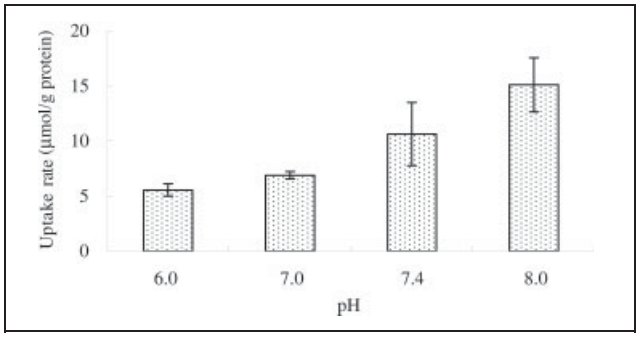

Fig. 1: Effects of pH on the uptake of zolmitriptan on Caco-2 cell monolayer apical sides. Uptake experiments with zolmitriptan 0.8 mmol · L^{-1} were performed at 37 °C for 2 h, n = 3

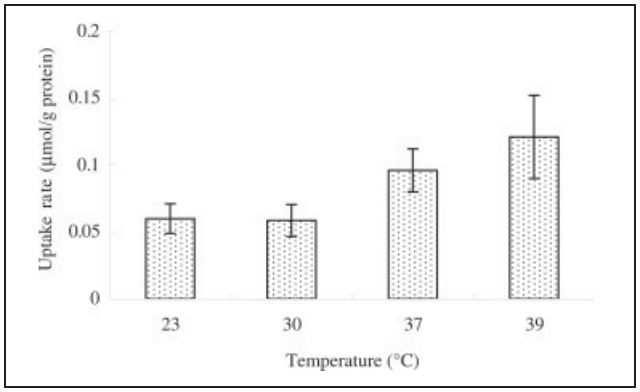

Fig. 2: Effect of temperature on uptake rate of zolmitriptan on Caco-2 cell monolayer apical sides. Uptake experiments with zolmitriptan 0.2 mmol · L^{-1} were performed in an environment of pH 7.4 for 2 h, n = 3

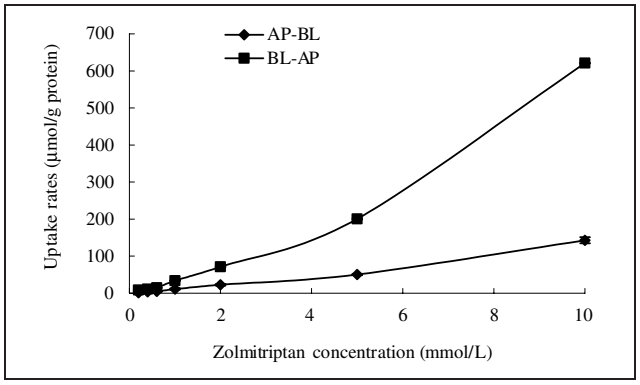

Fig. 3: Effect of the zolmitriptan concentration on Caco-2 cell uptake rates. The concentrations of zolmitriptan were 0.2, 0.4, 0.6, 1.0, 2.0, 5.0 and 10 mmol · L^{-1}, respectively, n = 3

rates of zolmitriptan on the basolateral side were 3–7 times higher than that on the apical side.

2.3. Effect of different drugs on zolmitriptan uptake

A series of experiments were done to address the nature of zolmitriptan uptake on both the apical and the basolateral side. The results shown in the Table indicated that verapamil, nimodipine, nifedipine, flunarizine, amiloride and sumatriptan significantly increased zolmitriptan uptake on the apical side, but the zolmitriptan uptake on the basolateral side showed some irregularity. Aspirin and propanolol had no significant effect to the zolmitriptan uptake at both sides. Propafenone significantly inhibited zolmitriptan uptake at both sides.

Table: Effect of different interaction drugs on zolmitriptan uptake

Compound	Uptake rate (μmol · g^{-1}-protein)			
	Zolmitriptan at apical side		Zolmitriptan at basal side	
Control	5.75 ± 0.35	100	12.20 ± 0.69	100
Verapamil	7.11 ± 0.89	124[a]	8.65 ± 0.46	71[a]
Nimodipine	9.99 ± 0.36	174[b]	14.76 ± 0.13	121[a]
Nifedipine	8.73 ± 0.19	152[b]	11.36 ± 0.28	93
Propranolol	6.33 ± 0.79	110	10.60 ± 1.20	87
Flunarizine	7.59 ± 0.24	132[b]	11.73 ± 0.54	96
Amiloride	7.21 ± 0.34	125[a]	14.22 ± 1.02	117[a]
Sumatriptan	6.66 ± 0.06	116[a]	13.61 ± 0.12	112[a]
Aspirin	5.77 ± 0.34	100	12.38 ± 0.81	101
Propafenone	3.57 ± 0.08	62[b]	4.54 ± 0.27	37[b]

Data are expressed as mean ± SD of three independent experiments. [a] Significantly different from the control ($p < 0.05$), [b] Significantly different from the control ($p < 0.01$). The concentration of zolmitriptan was 0.8 mmol · L^{-1}, and the concentration of the inhibitors was 0.5 mmol · L^{-1}, n = 3

3. Discussion

The human colon adenocarcinoma cell line, Caco-2 has been investigated as a useful alternative to animal models to study intestinal absorption of therapeutic agents including proteins, peptides, and oligo-nucleotides, and the transport and uptake studies observed in Caco-2 cells is probably correlated with the *in vivo* studies (Wang et al. 1997). The results indicated that the uptake of zolmitriptan significantly depended on the donor temperature and pH and showed a greater uptake at higher temperature and pH. As an alkalescence drug, free zolmitriptan increased with the increase in pH donor which may be the reason for a greater uptake. Although the zolmitriptan uptake was better at higher temperature and pH, considering the real environment of the small intestine, we designed the other experiments at 37 °C and pH 7.4.

In the concentration dependence experiments, the uptake rates of zolmitriptan on both sides increased with zolmitriptan concentrations increasing from 0.1 to 10 mmol · L^{-1}. No saturation was observed, but the uptake showed concave concentration dependency at high concentration. And the uptake rates of zolmitriptan on the basolateral side were 3–7 times higher than that on the apical side. These results indicated that some efflux mechanisms may be involved in the absorption of zolmitriptan.

P-Glycoprotein is one of the major active-transport proteins at the brush-border membrane of the human intestinal cell-line Caco-2. P-gp does limit drug absorption by extruding the drug from epithelial cells into the intestinal lumen. In order to investigate if P-gp is involved in the active uptake of zolmitriptan, verapamil (Racker et al. 1986), nimodipine (Zhang et al. 2003), and nifedipine (Kim et al. 1999), three well-known P-gp antagonist, were used to inhibit the uptake of zolmitriptan. All the three P-gp antagonists significantly increased the uptake of zolmitriptan on the apical side, and suggested that P-gp is involved in the active uptake of zolmitriptan. But it was confusing that verapamil deduced and nifedipine did not increase the uptake rate of zolmitriptan on the basolateral side. The reason was unclear. In the concentration dependence experiments, the uptake rates of zolmitriptan on both sides showed concave concentration dependency at high concentration. One of the reasons for that may be that some of the P-gp receptor was saturated by zolmitriptan at high concentration.

Zolmitriptan is used in the acute oral treatment of migraine, and it is likely to be co-administered with other drugs, such as propranolol (Peck et al. 1997), flunarizine (Lewis et al. 2004), sumatriptan (Lewis et al. 2004), aspirin (Katsarava and Limmroth 2004) and propafenone. As an attempt to explain the *in vivo* interactions, we investigated the possible *in vitro* uptake interactions between zolmitriptan and some inhibitors at cell level. Flunarizine significantly increased the apical side zolmitriptan uptake in Caco-2 cell monolayers. No reports indicate it flunarizine is a substrate or inhibitor of P-gp. The human intestinal cell line Caco-2 constitutively expresses such drug transporters as peptide transporter, P-gp, organic cation transporter and organic aniotransporter (Tsuji and Takanaga 1994), so flunarizine may inhibit the other drug transporter leading to the increase of zolmitriptan uptake. Sumatriptan, a P-gp substrate (Evans 2003), also significantly increased zolmitriptan uptake on both the Caco-2 monolayer sides. It was reported that propranolol increased mean zolmitriptan C_{max} and AUC by 56% and 37% in humans, respectively (Peck et al. 1997). In a previous study we found that drug−drug metabolism interaction between zomitriptan and propranolol may be one of the reasons leading to the increase of C_{max} and AUC of zolmitriptan (Yu et al. 2003). The present result indicated that propranolol had no significant effect on the absorption of zolmitriptan *in vitro*. Amiloride significantly increased the uptake of zolmitriptan, hence the Na^+-H^+ antiporter may have some involvement (Crowe and Wong 2004). Propafenone significantly reduced the zolmitriptan uptake on both sides. In the previous study, propafenone was shown to modulate P-gp ATPase activity (Diethart and Gerhard 1999). Lower concentrations stimulated the activity of P-gp ATPase, as the result increasing the efflux of the drug and reduced the uptake of zolmitriptan.

So absorption interactions should be considered when P-gp substrates or inhibitors, Na^+-H^+ exchange inhibitors, P-gp ATPase agonists or inhibitors are co-administered with zomitriptan in clinical practice.

4. Experimental

4.1. Materials

Zolmitriptan, sumatriptan succinate and aspirin were provided by Department of Chemistry (Zhejiang University, Hangzhou, China). Verapamil hydrochloride, propranolol hydrochloride, amiloride hydrochloride, nimodipine, nifedipine, flunarizine, propafenone hydrochloride and fluorescein were purchased from Sigma (St. Louis, Mo, USA). All other chemicals were regent grade. The stock solutions of zolmitriptan and other compounds were made in DMSO. The concentration of DMSO in the final solutions was 0.5%, a concentration which 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 medium (DMEM), fetal bovine serum (FBS), non-essential amino acids (NEAA), L-glutamine, trypsin and antibiotic mixture (10,000 IU $\cdot$ ml^{-1} penicillin G, 10,000 µg $\cdot$ ml^{-1} streptomycin) were purchased from Gibco (Life Technologies, Paisley, Scotland, UK). Twelve-well Transwells were purchased from Corning Costar Corporation (Costar, Cambridge, MA, USA).

4.2. Cell culture

Caco-2 cells were grown in DMEM supplemented with 10% FBS, 1% NEAA and 5% antibiotic-antimycotic solution at 37 °C in culture flasks (Thermo Electron Corporation) in a humidified air-5% CO_2 atmosphere. Cells were harvested with trypsin (0.25%)-EDTA (1 mmol $\cdot$ L^{-1}) and seeded onto polycarbonate filters (0.3 µm $\cdot$ 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 used for the following experiments. All cells were used in this study between passages 50–70. The integrity of the cell monolayer was checked by measuring the transcellular permeability of fluorescein and the transepithelial electrical resistance (TEER) across the monolayers. The normal TEER values ranged from 400–600 Ω $\cdot$ cm^{-2}, and the permeability coefficient (P_{app}) of fluorescein flux (AP-BL) was less than 0.5×10^{-6} cm $\cdot$ s^{-1}.

4.3. Uptake studies

Cell monolayers were rinsed and incubated with HBSS (containing 25 mmol $\cdot$ L^{-1} HEPES, pH 7.4) at 37 °C for 30 min prior to the start of the experiment. TEER was measured after 30 min.

4.3.1. Effect of temperature

Zolmitriptan solution (0.5 ml, 0.2 mmol $\cdot$ L^{-1}) prepared in HBSS (pH 7.4) was added to the apical side. Solution and the plates were incubated at 23, 30, 37 and 39 °C separately for 2 h. The cell monolayers were washed four times with ice-cold HBSS after incubation. Then, cells were removed by treatment with 0.3 ml trypsin (0.25%)-EDTA (1 mmol $\cdot$ L^{-1}) and homogenized by supersonics. The amount of zolmitriptan was then analyzed by HPLC. The amount of protein in the cell homogenate was measured according to Lowry (1951). Uptake is expressed as µmol $\cdot$ g^{-1}-protein.

4.3.2. Effect of pH

Zolmitriptan solutions (0.5 ml, 0.8 mmol $\cdot$ L^{-1}) prepared in HBSS with pH 6.0, 7.0, 7.4 and 8.0 separately were added to the apical side, and the plates were incubated at 37 °C for 2 h. HCl and NaOH were used to adjust the pH of HBSS. Then, cell monolayers were treatmented with the same way as above.

4.3.3. Concentration-dependency uptake of zolmitriptan on Caco-2 cell monolayer

HBSS solution (pH 7.4) containing zolmitriptan in different concentrations (0.2, 0.4, 0.6, 1.0, 2.0, 5.0 and 10 mmol $\cdot$ L^{-1}) was added on either the apical (0.5 ml) or the basolateral (1.5 ml) side of the inserts, while the receiving chamber contained the corresponding volume of HBSS solution and the plates were incubated at 37 °C for 2 h. Then, cell monolayers were treated the same way as above.

4.3.4. Competition experiments

The zolmitriptan solution (0.8 mmol $\cdot$ L^{-1}) prepared in HBSS (pH 7.4) was added either on the apical (0.5 ml) or basolateral (1.5 ml) side of the inserts, but the possible interaction drugs (0.5 mmol $\cdot$ L^{-1}) were all added to the apical side. After incubated at 37 °C for 2 h, cell monolayers were treated the same way as above.

4.4. HPLC assay

The cell homogenate (250 µl) was mixed with K_2HPO_4 (100 µl, 10 mM, pH 9.4) and chloroform/isopropanol (75/25, v/v). Then the mixture was vortexed and centrifuged (10,000 g) for 5 min. After that, the chloroform/isopropanol layer was separated and dried under vacuum. The residue was dissolved in 100 µl of mobile phase, and 20 µl was injected into the HPLC system.

All the samples described above were analyzed by reversed phase HPLC, 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 mol $\cdot$ L^{-1}KH_2PO_4 (pH 7.5) (25/75, v/v) with a flow rate of 1.0 ml $\cdot$ min^{-1}. The detection wavelength was 229 nm, and the retention time of zolmitriptan was about 7 min.

4.5. Data analysis

Data are expressed as mean ± SD. The *t*-test was used for statistical analysis and statistical significance was defined as $p < 0.05$ or $p < 0.01$.

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