Effect of Cimetidine on the Transport of Quinolone Antibiotics in Caco-2 Cell monolayers

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Abstract – Cimetidine, a substrate for P-glycoprotein (P-gp), is a well known drug interacting with a variety of drugs and results in alteration of pharmacokinetic parameters by concomitant administration. The aim of present study was to investigate whether cimetidine affects the transport of various quinolone antibiotics in human colorectal cancer cell line (Caco-2) system which has been typically used to investigate drug transport via P-gp. The apparent permeability coefficients (Papp) value of 9 quinolone antibiotics in the co-treatment with cimetidine was examined. Apical to basolateral (AP-to-BL) transport of fleroxacin in the co-treatment with cimetidine was increased to 1.5-fold (p<0.01) compared with that of fleroxacin alone, whereas basolateral to apical (BL-to-AP) transport of fleroxacin was decreased to 0.83-fold significantly (p<0.05). Ofloxacin was decreased to 0.8-fold (p<0.01) and 0.72-fold (p<0.01) significantly in AP-to-BL and BL-to-AP direction, respectively by cimetidine co-treatment. The Papp values of gatifloxacin, moxifloxacin, ciprofloxacin and rufoxacin also were changed by cimetidine. These results have a potential that cimetidine influences on the pharmacokinetics of quinolone antibiotics. It suggests that careful drug monitoring and dosage adjustment may be necessary during the co-administration of quinolone antibiotics with cimetidine.

Key words □ Cimetidine, P-glycoprotein, Quinolone antibiotics, Caco-2, Apparent permeability coefficients

INTRODUCTION

Most of drugs are administered orally and absorbed across the intestinal epithelium, where exists a variety of transporters including a P-glycoprotein (P-gp) (Ayrton and Morgan et al., 2001). P-gp, called MDR for multidrug resistance, is a trans-membrane protein and acts as an efflux pump ATP-dependent (Juliano et al., 1976). P-gp is encoded by the MDR1 gene in humans, whereas is encoded by the mdr1a and mdr1b genes in mice (Schinkel et al., 1997; Gottesman and Pastan et al., 1998). It pumps many drugs outside cells, decreases their intracellular concentrations (Karner et al., 1983), and consequently limits intestinal drug absorption (Suzuki and Sugiyama et al., 2000; Ayrton and Morgan et al., 2001). P-gp is known to be a barrier to the transport for many drugs (Balayssac et al., 2005) and then may be an important factor influencing in the drug absorption, despite it acts as a protective barrier for excluding potentially toxicant (Germann et al., 1993).

Caco-2 cell, human colorectal adenocarcinoma cell line, has special properties that is differentiated well and forms a polarized monolayer of intestinal epithelium. Caco-2 monolayer system is widely used as a good model to estimate intestinal drug permeability (Artursson and Karlsson, 1991; Rubis et al., 1993). Caco-2 cell has a variety of transporters including P-gp (Hunter et al., 1993), which can be confirmed by expression of mRNA and protein in Caco-2 cell (Nanum et al., 2006).

Quinolone antibiotics, extensively prescribed to treat diverse bacterial infections, have bioavailability of about 50~95% (Neu et al., 1992; Weidekamm et al., 1987; Wise et al., 1984). Previous studies reported that oral co-administration of quinolone antibiotics and some antacids such as Al³⁺ or Mg²⁺ decreases its absorption and bioavailability (Jaehde et al., 1994;
which could be explained by chelate complexes formation of these drugs (Shimada et al., 1992). The alteration of absorptive rate by concomitant administration results in connected to pharmacokinetic changes and eventually is due to result in therapeutic failure or adverse drug reaction administration. Cimetidine, a well known histamine H2-receptor antagonist, causes to interact other drugs and results in alteration of pharmacokinetic parameters by co-administration (Karyekar et al., 2004; Kolawole et al., 2006; Kosoglou et al., 2000), although cimetidine is often prescribed with other drugs for reducing acid secretion. It has not been well studied whether cimetidine alters the absorption of quinolone antibiotics or not. Therefore, it is essential to investigate the pharmacokinetic effect on co-administration of cimetidine and quinolone antibiotics in order to provide adequate medication information for doctors and safe usage of drugs for patients. Cimetidine is absorbed passively across the intestinal epithelium (Can et al., 1993) and is associated with P-gp in drug absorption (Pan et al., 1994). According to Pan B.H. et al., 1994, the transport of cimetidine was increased in BL-to-AP by P-gp, showing that cimetidine is a substrate for P-gp. In this study, we evaluated the effect of quinolone antibiotics in the co-treatment with cimetidine using in vitro Caco-2 cell permeability system in which P-gp acts as a representative drug efflux transporter.

**MATERIALS AND METHODS**

**Materials**

We obtained 9 drugs from LG Life Sciences, etc and obtained Caco-2 cells from American Type Culture Collection (Rockville, MD, USA). We purchased Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA and penicillin/streptomycin from Gibco BRL (Grand Island, NY, USA); L-glutamine, sodium bicarbonate, nonessential amino acid, Hank's balanced salts solution (HBSS) and sodium pyruvate from Sigma (Milwaukee, WI, USA); glucose from Calbiochem (La Jolla, CA, USA); and penicillin/streptomycin, and ImM sodium pyruvate. Confluent cell monolayer was subcultured every 7 days by treatment with 0.25% trypsin containing ImM EDTA. Caco-2 cells were seeded at the density of 3.2×10^5 cells/cm^2 in 12-well plates on transwell polycarbonate filters. Cells were given fresh medium every 2 or 4 days and grown fully differentiated until 21 days, and all experiments were conducted between 21 and 22 days. Cells were used between passage numbers 30 and 50, and initial transepithelial electrical resistance (TEER) values of Caco-2 cell monolayer were measured with a Millicell-ERS Voltmeter (Millipore, Billerica, MA, USA) and those of higher than 400Ω·cm^2 were used (Klingberg et al., 2005).

Caco-2 cell system suitability

*In vitro* Caco-2 cell permeability system suitability was performed according to Jung S.J. et al.

**Transport study**

Caco-2 cell monolayers were preconditioned by incubating with HBSS (pH 7.4) consisting of 1.3 mM CaCl_2, 5.4 mM KCl, 0.44 mM K_2HPO_4, 0.49 mM MgCl_2, 0.1mM MgSO_4, 137 mM NaCl, 0.34 mM Na_2HPO_4, 5.5 mM D-glucose, and 42 mM NaHCO_3 at 37°C for 30min. Quinolone antibiotics and cimetidine of the concentration corresponding to 0.1 times of the highest dose strength dissolved in 250mL of buffer were used in the transport study. For apical to basolateral (AP-to-BL) experiments, the solution was placed on the apical side of the cells, and samples were taken from the basolateral side. At many time points, samples were collected from the other side of the cell monolayers for quantification. All transport studies were performed at 37°C. The samples were analyzed by HPLC system (Waters, Milford, MA, USA) consisting of photo diode array detector (Waters 299), binary HPLC pump (Waters 1525) and autosampler (Waters 717 plus) to determine the drug concentration. A 5 µm 250 mm×4.6 mm UG120 C_18 column (Shiseido, Tokyo, Japan) was used along with a mobile phase, flow rate and injection volume displayed in Table I.

Permeability coefficient (P_app) was calculated according to the following equation:

\[ P_{app} = \frac{(dQ/dt)}{(C_0×A)} \text{(cm/sec)} \]

Where dQ/dt is the permeability rate C_0 is the initial concentration of the solution in the donor compartment, and A is the surface area of the membrane. The efflux rate (dQ/dt)/(1×A)
was determined by plotting the amount transported per unit area as a function of time and determining the slope of the line using linear regression. We used antipyrine (0.8 mg/mL) as internal standard of high transport and calculated $P_{\text{app}}$ ratio by of each drug and $P_{\text{app}}$ of antipyrine to compare with drug transport. Each result was performed in $n=4$ and the data represent the mean ± standard deviation.

**Statistical analysis**

Data obtained from Caco-2 cell permeability tests was expressed as mean ± standard deviation in tables and figures. Statistical analysis for significant differences was calculated using the unpaired student’s t-test. Differences were considered significant at a $p$-value of 0.05 or less.

**RESULT**

**Transepithelial electrical resistance measurements**

To determine an appreciate number of cell on plate and to confirm the morphologic and physiologic cell condition and confluence, Caco-2 cells were seeded on transwell in various cell concentration. In $2.4\times10^5$ cell/mL and $3.2\times10^5$ cell/mL, TEER values increased until day 17~19 and then reached a plateau that lasted until day 21. Because TEER values of $3.2\times10^5$ cell/mL were $500~600\Omega \cdot \text{cm}^2$ at approximately day 20 (Behrens and Kissel et al., 2003; Geisen et al., 2006), this number of cell was used for transport study, which was conducted at day 21 (Hosoya et al., 1996). In $1.6\times10^5$ cell/mL and $6.4\times10^5$ cell/mL, TEER values increased until day 21 and thereafter decreased (Fig. 1).

**Drug transport experiments**

To investigate the effect of cimetidine on quinolone antibiotics, we studied the transport of co-treatment of each quinolone antibiotics and cimetidine in Caco-2 cell medium. The result of the transport is shown in Table II. We found that the apparent permeability coefficient ($P_{\text{app}}$) value of gatifloxacin in the co-treatment group of gatifloxacin and cimetidine was significantly increased ($p<0.05$) in the basolateral-to-apical (BL-to-AP) direction compared with that of cimetidine alone. Apical-to-basolateral (AP-to-BL) drug transport of moxifloxacin was significantly increased ($p<0.01$). The $P_{\text{app}}$ value of fleroxacin with cimetidine in the AP-to-BL direction, was decreased from $13.37 \pm 0.57$ to $8.89 \pm 1.26$ ($p<0.01$) that of fleroxacin alone, whereas that of fleroxacin with cimetidine in the BL-to-AP direction, $10.45 \pm 0.45$ was increased to $12.56 \pm 1.73$ ($p<0.05$). It was significantly increased 1.5-fold in the AP-to-BL transport and was decreased 0.83-fold in the BL-to-AP transport.

**Table I. HPLC analytical condition of drugs for permeability.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mobile phase</th>
<th>Flow rate (mL/min)</th>
<th>Detector/λ(nm),</th>
<th>Inj. vol. (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gatifloxacin</td>
<td>20: 80: 1$^a$</td>
<td>1.0</td>
<td>UV/293,</td>
<td>20</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>20: 80: 1$^a$</td>
<td>1.0</td>
<td>UV/295</td>
<td>20</td>
</tr>
<tr>
<td>Lomefloxacin</td>
<td>20: 80: 1$^a$</td>
<td>1.0</td>
<td>UV/287</td>
<td>20</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>20: 80: 1$^a$</td>
<td>1.0</td>
<td>UV/298</td>
<td>20</td>
</tr>
<tr>
<td>Fleroxacin</td>
<td>20: 80: 1$^a$</td>
<td>1.0</td>
<td>UV/287</td>
<td>20</td>
</tr>
<tr>
<td>Enoxacin</td>
<td>30: 70$^b$</td>
<td>1.0</td>
<td>UV/269</td>
<td>20</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>30: 70$^b$</td>
<td>1.0</td>
<td>UV/287</td>
<td>20</td>
</tr>
<tr>
<td>Rufloxacin</td>
<td>30: 70$^b$</td>
<td>1.0</td>
<td>UV/246</td>
<td>20</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>30: 70$^b$</td>
<td>1.0</td>
<td>UV/292</td>
<td>20</td>
</tr>
</tbody>
</table>

$^a$Mobile phase composition – acetonitrile:water:glacial acetic acid

$^b$Mobile phase composition – methanol:water(0.03% phosphoric acid)

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![Fig. 1. TEER value after seeding in Caco-2 cell by various cell concentrations. TEER of the monolayers was measured at 2, 6, 8, 9, 12, 14, 16, 19, 21, 23 and 26 day after seeding at a concentration of $1.6\times10^5$ (●), $2.4\times10^5$ (■), $3.2\times10^5$ (▲), $6.4\times10^5$ (▲) cell/mL. TEER was measured in both apical and basolateral at the same time and the value is the average of three measurements.](image-url)
The P_app value of ciprofloxacin with co-treatment of cimetidine in the AP-to-BL direction was significantly higher approximately 2 times (p<0.05) than that of cimetidine alone. The BL-to-AP rufloxacin transport was significantly increased from 7.28 ± 0.23 to 10.71 ± 0.50 (p<0.01) in the BL-to-AP direction. It was significantly reduced 0.8-fold and 0.72-fold in the AP-to-BL and BL-to-AP direction, respectively. Quinolone antibiotics with significant changes by cimetidine in Caco-2 cell monolayer system were shown in Table III. Moxifloxacin, fleroxacin and ciprofloxacin were significantly increased permeability AP-to-BL, otherwise rufloxacin was significantly decreased AP-to-BL by co-administration with cimetidine.

Fleroxacin of those drugs had a growth transport in the uptake direction (AP-to-BL), while a reduction transport in the efflux direction (BL-to-AP). Consequently, we estimated that the absorption rate of fleroxacin in the co-administration may be higher than that in the fleroxacin alone resulting from an increase in uptake transport and a decrease in efflux transport.

**DISCUSSION**

P-gp, one of the widely famous transporters, plays an essential role in limiting the intestinal absorption of a variety of drugs. Concomitant administration of drugs related to transporters would alter drug pharmacokinetics by changing bioavailability and organ uptake, if more serious cases happen, may cause adverse drug reaction or toxicity. The interaction of quinidine and digoxin is a representative example. According to Pedersen et al., 1983, quinidine that is a P-gp inhibitor, increased the bioavailability and plasma concentration of digoxin, a P-gp substrate, and eventually resulted in change the pharmacokinetics of digoxin. It may need to diminish the dose contents of digoxin dose to protect the toxicity of digoxin. Pan et al., 1994 and Collett et al., 1998 reported the BL-to-AP transport of cimetidine was increased. These means that cimetidine is a substrate for P-gp or has the potential as a substrate of P-gp. As known, substrates have potential as a competitive or noncompetitive inhibitor with other substrates. Although some researchers have shown that absorption of cimetidine can be a effected by other P-gp inhibitors including verapamil and psc-833, there are few information on whether or not it can alter the absorption of other P-gp substrates. Therefore, in this study we have focused on the effect of cimetidine and quinolone antibiotics, clinically relevant with the drug, on transporting related P-gp.

As shown in Table II, the transports of quinolone antibiotics were significantly affected by cimetidine. But these quinolone antibiotics showed various results by co-administration with cimetidine. In previous our study, quinolone antibiotics were examined permeability in Caco-2 system. The permeability of the then was also different among them (data not shown). These results suggest that cimetidine may alter the absorption rate of quinolone antibiotics in vivo when is concomitantly administered. This is consistent with previous report, Foote and Halstenson, 1998. They pre-administrated cimetidine prior to dosing with ofloxacin in rats, monitored the plasma concentration of ofloxacin, and found that pharmacokinetic parameters of ofloxacin in ofloxacin plus cimetidine group were changed

<table>
<thead>
<tr>
<th>Drug</th>
<th>P_app (AP-to-BL)</th>
<th>P_app (AP-to-BL)</th>
<th>P_app (BL-to-AP)</th>
<th>P_app (BL-to-AP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gatifloxacin</td>
<td>5.29±0.90</td>
<td>5.54±0.71</td>
<td>5.25±0.71</td>
<td>6.22±0.73</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>5.06±1.38</td>
<td>7.63±2.18</td>
<td>7.86±0.82</td>
<td>8.21±0.71</td>
</tr>
<tr>
<td>Lomefloxacin</td>
<td>3.41±2.24</td>
<td>5.19±0.10</td>
<td>5.23±1.50</td>
<td>5.2±0.73</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>10.60±0.57</td>
<td>11.94±0.56</td>
<td>15.43±0.65</td>
<td>16.56±2.17</td>
</tr>
<tr>
<td>Fleroxacin</td>
<td>8.89±1.26</td>
<td>13.37±0.57</td>
<td>12.56±1.73</td>
<td>10.45±0.45</td>
</tr>
<tr>
<td>Enoxacin</td>
<td>8.56±2.39</td>
<td>6.86±1.58</td>
<td>2.81±1.62</td>
<td>4.30±0.44</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2.55±0.25</td>
<td>4.47±1.22</td>
<td>3.49±0.27</td>
<td>3.9±0.68</td>
</tr>
<tr>
<td>Rufloxacin</td>
<td>18.07±4.10</td>
<td>18.09±0.28</td>
<td>7.28±0.23</td>
<td>10.71±0.50</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>5.56±0.16</td>
<td>4.46±0.28</td>
<td>13.51±0.59</td>
<td>9.74±0.80</td>
</tr>
</tbody>
</table>

*Drugs concentrations corresponding to 0.1 times of the highest dose strength dissolved in 250 mL of buffer (CDER, FDA, August, 2000).*
Table III. Significant effect of quinolone antibiotics on co-treatment of cimetidine.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Transport of quinolone antibiotics</th>
<th>AP-to-BL</th>
<th>BL-to-AP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moxifloxacin</td>
<td>+</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Fleroxacin</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>+</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Rufloxacin</td>
<td>NS</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Olofoxacin</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a - * represents significant increase of P_{app} value compared with treatment group of antibiotics alone  
b - - represents significant decrease P_{app} value compared with treatment group of antibiotics alone  
c - NS represents no significant

significantly compared with ofloxacim alone group. The transport effect of cimetidine on quinolone antibiotics resulted in various change with direction and degree. Olofoxacin showed the same pattern in both AP-to-BL and BL-to-AP directions, whereas fleroxacin displayed the opposite pattern. Moxifloxacin and ciprofloxacin showed the absorption in the AP-to-BL, whereas rufloxacin increased in the BL-to-AP transport. These results suggest that this alteration may be possible to be different depending on drug type of quinolone antibiotics.

The P_{app} value of fleroxacin with cimetidine was increased to 1.5-fold \((p<0.01)\) in the uptake transport direction (AP-to-BL) than that of fleroxacin alone, while decreased to 0.83-fold \((p<0.05)\) in the eflux direction (BL-to-AP). Based on this data, it is estimated that co-administration of fleroxacin and cimetidine increases the absorption rate of fleroxacin. The P_{app} value of moxifloxacin was 1.13 times greater by co-treatment with cimetidine in the uptake transport direction than moxifloxacin alone group. In spite of no significant effect in the eflux direction, the absorption of these two drugs may increase by co-administration with cimetidine. It is assumed that absorption of quinolone antibiotics may be changed by cimetidine. It seems to need more studies to elucidate the mechanism of quinolone antibiotics transport via P-pg with cimetidine.

In conclusion, cimetidine influenced significantly on the transport of quinolone antibiotics in Caco-2 cells. This suggests a change of quinolone antibiotics absorption by co-administration of cimetidine.

REFERENCES


