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

Seon Hwa Kim[1], Seo Jeong Jung[2], So Young Um[1], Mi Ae Na[3], Min Jin Choi[1],
Myeon Woo Chung[1], Hye Young Oh[1]*

[1]*Drug MetabolismTeam, Department of Pharmacology, National Institute of Toxicological Research,
Korea Food and Drug Administration, 5 Nokbun-dong, Eunpyung-gu, Seoul, Korea*

[2]*Center for Food & Drug Anaylsis, Busan KFDA, 123-7 Yong Dang-dong, Nam-gu, Busan, Korea*

[3]*Testing & Analysis Team, Daejeon KFDA, 120, Seonsa-ro, Seo-gu, Daejeon, Korea*

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 (P_{app}) 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 P_{app} values of gatifloxacin, moxifloxacin, ciprofloxacin and rufloxacin 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 administrated 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 transmembrane protein and acts as 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.*, 1993). It pumps many drugs outside cells, decreases their intracellular concentrations (Kartner *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 resisted

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; Rubas *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 (Narumi *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 Al^{3+} or Mg^{2+} decreases its absorption and bioavailability (Jaehde *et al.*, 1994;

*Corresponding author
Tel: +82-2-380-1774,   Fax: +82-2-380-1776
E-mail: hyeongoh@kfda.go.kr

Lazzaroni *et al.*, 1993), 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 H_2-receptor antagonist, causes to interact other drugs and results in alteration of pharmacokinetic parameters by co-administration (Karyekar *et al.*, 2004; Kolawole *et al.*, 2000; 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 (Gan *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 (Milwakee, WI, USA), and transwell polycarbonate filters from Costar Corning (NY, USA; diameter 12 mm, pore size 0.4 μm). HPLC grade acetonitrile was purchased from J.T.Baker (Deventer, The Netherlands). Water was purified with and ultimate reverse osmosis system (Barnstead, Dubuque, USA). And all of the other reagents were of analytical or HPLC grade.

Caco-2 cell permeability test
Cell culture
Caco-2 (ATCC HTB37) were grown in 75 cm² T-flask at 37

°C in the atmosphere of 5% CO_2 / 95% air and 90% relative humidity in DMEM supplemented with 10% FBS, 1% nonessential amino acids, 100 Unit/mL penicillin and 100 μg/mL streptomycin, and 1mM sodium pyruvate. Confluent cell monolayer was subcultured every 7 days by treatment with 0.25% trypsin containing 1mM EDTA. Caco-2 cells were seeded at the density of 3.2×10^5 cells/cm² 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 Voltohmmeter (Milllipore, Billerica, MA, USA) and those of higher than 400Ω · cm² 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 4.2 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 2996), 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:

$$P_{app} = (dQ/dt)/(C_0 \times A) \text{ (cm/sec)}$$

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 $(dQ/dt)/(1 \times A)$

Table I. HPLC analytical condition of drugs for permeability.

Drug	Mobile phase	Flow rate (mL/min)	Detector/ë(nm),	Inj. vol. (μl)
Gatifloxacin	20: 80: 1[a]	1.0	UV/293,	20
Levofloxacin	20: 80: 1[a]	1.0	UV/295	20
Lomefloxacin	20: 80: 1[a]	1.0	UV/287	20
Moxifloxacin	20: 80: 1[a]	1.0	UV/298	20
Fleroxacin	20: 80: 1[a]	1.0	UV/287	20
Enoxacin	30: 70[b]	1.0	UV/269	20
Ciprofloxacin	30: 70[b]	1.0	UV/287	20
Rufloxacin	30: 70[b]	1.0	UV/246	20
Ofloxacin	30: 70[b]	1.0	UV/292	20

[a]Mobile phase composition – acetonitrile:water:glacial acetic acid
[b]Mobile phase composition – methanol:water(0.03% phosphoric acid)

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_{app} ratio by of each drug and P_{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 2.4×10^5 cell/mL and 3.2×10^5 cell/mL, TEER values increased until day 17~19 and then reached a plateau that lasted until day 21. Because TEER values of 3.2×10^5 cell/mL were 500~600Ω · cm² 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 1.6×10^5 cell/mL and 6.4×105 cell/mL, TEER values increased until day 21 and thereafter decreased (Fig. 1).

Drug transport experiments

To investigate the effect of cimetidine on quinolone antibiot-

ics, 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_{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_{app} value of fleroxacin with cimetidine in the AP-to-BL direction, was decreased from 13.37 ± 0.57 to 8.89 ± 1.26 ($p<0.01$) that of fleroxacin alone, whereas that of fleroxacin with cimetidine in the BL-to-AP direction, 10.45 ± 0.45 was increased to 12.56 ± 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.
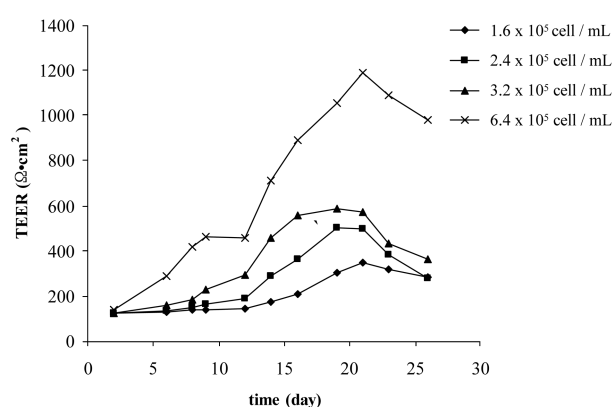

Fig. 1. TEER value after seeding in Caco-2 cell by various cell concentrations. TEER of the monolayers was measured at 2, 6, 8, 9, 12, 14, 16, 19, 21, 23 and 26 day after seeding at a concentration of 1.6×10^5 (◆), 2.4×10^5 (■), 3.2×10^5 (▲), 6.4×10^5 (×) cell/mL. TEER was measured in both apical and basolateral at the same time and the value is the average of three measurements.

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) that in the cimetidine co-treatment group. The P_{app} value of ofloxacin was decreased by co-treatment with cimetidine from 5.56 ± 0.16 to 4.46 ± 0.28 (p< 0.01) in the AP-to-BL direction and from 13.51 ± 0.59 to 9.74 ± 0.80 (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 bio-availability 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 affected 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 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 II. Effect of co-treatment of cimetidine on Caco-2 cell permeability of quinolone antibiotics[a] (P_{app}= $\times 10^{-6}$ cm/sec, n=4, *p<0.05, **p <0.01).

Drug	Cimetidine			
	-	+	-	+
	P_{app} (AP-to-BL)	P_{app} (AP-to-BL)	P_{app} (BL-to-AP)	P_{app} (BL-to-AP)
Gatifloxacin	5.29±0.90	5.54±0.71	5.25±0.71	6.22±0.73*
Levofloxacin	5.06±1.38	7.63±2.18	7.86±0.82	8.21±0.71
Lomefloxacin	3.41±2.24	5.19±0.10	5.23±1.50	5.21±0.73
Moxifloxacin	10.60±0.57	11.94±0.56**	15.43±0.65	16.56±2.17
Fleroxacin	8.89±1.26	13.37±0.57**	12.56±1.73	10.45±0.45*
Enoxacin	8.56±2.39	6.86±1.58	2.81±1.62	4.30±0.44
Ciprofloxacin	2.55±0.25	4.47±1.22*	3.49±0.27	3.95±0.68
Rufloxacin	18.07±4.10	18.09±0.28	7.28±0.23	10.71±0.50**
Ofloxacin	5.56±0.16	4.46±0.28**	13.51±0.59	9.74±0.80**

[a]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.

Drug	Transport of quinolone antibiotics	
	AP-to-BL	BL-to-AP
Moxifloxacin	+	NS
Fleroxacin	+	−
Ciprofloxacin	+	NS
Rufloxacin	NS	+
Ofloxacin	−	−

[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 ofloxacin alone group. The transport effect of cimetidine on quinolone antibiotics resulted in various change with direction and degree. Ofloxacin showed the same pattern in both AP-to-BL and BL-to-AP directions, whereas fleroxacin displayed the opposite pattern. Moxifloxacin and ciprofloxacin increased in the AP-to-BL transport, whereas rufloxacin increased in the BL-to-AP transport. These results suggest that this alteration may be possible to be different dependently 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 efflux direction (BL-to-AP). Based on this data, it is estimated that co-administration of fleroxacin and cimetidine makes the absorption rate of fleroxacin increase. 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 efflux 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 quiolone antibiotics transport via P-pg with cimetidine.

In conclusion, cimetidine influenced significantly on the transport of quinolone antibiotics in Caco-2 cell model. This suggests absorption of quinolone antibiotics may be changed by co-administration of cimetidine.

REFERENCES

Artursson P., Karlsson J. (1991) Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. *Biochem. Biophys. Res. Commun.* 175, pp. 880-885

Ayrton A., Morgan P. (2001) Role of transport proteins in drug absorption, distribution and excretion. *Xenobiotica.* 31, pp. 469-497

Balayssac D., Authier N, Cayre A. Coudore F. (2005) Does inhibition of P-glycoprotein lead to drug-drug interactions? *Toxicol Lett.* 156, pp. 319-329.

Behrens I., Kissel T. (2003) Do cell culture conditions influence the carrier-mediated transport of peptides in Caco-2 cell monolayers? *Eur J Pharm Sci.* 19, pp. 433-442

Collett A., Higgs N. B., Sims E., Rowland M., Warhurst G. (1998) Modulation of the permeability of H_2 receptor antagonists cimetidine and ranitidine by P-glycoprotein in rat intestine and the human colone cell line Caco-2. *J. Pharmacol. Exp. Ther.*, 288, pp. 171-178

Chetan S. Karyekar, M.D., Ph.D., Natalie D. Eddington, Ph.D., Tushar S. Garimella, M.S., Paul O. Gubbins, Pharm.D., Thomas C. Dowling, Pharm.D., Ph.D. (2004) Evaluation of P-glycoprotein-Mediated Renal Drug Interactions in an MDR1-MDCK Model. *Pharmacother.* 23(4), pp. 436-442

Foote E.F. and Halstenson C.E. (1998) Effects of probenecid and cimetidine on renal disposition of ofloxacin in rats, *Antimicrob. Agents Cehmother.* 42, pp. 456-458

Gan L.S., Hsyu P.H., Pritchard J.F., Thakker D. (1993) Mechanism of intestinal absorption of ranitidine and ondansetron: Transport across Caco-2 monolayers. *Pharm. Res.* 10, pp. 1722-1725

Geisen P., McColm J.R., King B.M., Hartnett M.E. (2006) Characterization of barrier properties and inducible VEGF expression of several types of retinal pigment epithelium in medium-term culture. *Curr Eye Res.* 31, pp. 739-748.

Germann U.A., Pastan I., Gottesman M.M. (1993) P-glycoproteins: Mediators of multidrug resistance. Semin. *Cell Bil.* 4, pp. 63-76

Gottesman, M.M., Pastan I. (1993) Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu. Rev. Biochem.* 62, pp. 385-427

Hosoya K.I., Kim K.J., Lee V.H. (1996) Age-dependent expression of P-glycoprotein gp170 in Caco-2 cell monolayers. *Pharm. Res.* 13, pp. 885-890

Hunter J., Jepson M.A., Tsuruo T., Simmons N.L., Hirst B.H. (1993) Functional expression of P-glycoprotein in apical membranes of human intestinal Caco-2 cells. Kinetics of vinblastine secretion and interaction with modulators. *J. Biol. Chem.* 268, pp. 14991-14997

Jaehde U., Sorgel F., Stephan U., Schunack W. (1994) Effect of an antacid containing magnesium and aluminum on absorption, metabolism, and mechanism of renal elimination of pefloxacin in humans. *Antimicrob. Agents Cehmother.* 38, pp. 1129-1133

Juliano RL, Ling V. (1976) A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim Biophys Acta.* 455, pp. 152-162

Jung S.J., Choi S.O., Um S.Y., Kim J.I., Choo H.Y., Choi S.Y., Chung S.Y. (2006) Prediction of the permeability of drugs through study on quantitative structure-permeability relationship. *J. Pharm. Biomed. Anal.* 41, pp. 469-475.

Kartner N., Riodan J.R., Ling V. (1983) Cell surface P-glycoprotein associated with multidrug resistance in mammalian cell lines. *Science* 221, pp. 1285-1288

Karyekar C.S., Eddington N.D., Briglia A., Gubbins P.O., Dowling T.C. (2004) Renal interaction between itraconazole and cimetidine. *J. Clin. Pharmacol.* 44, pp. 919-927

Klingberg T.D, Pedersen M.H., Cencic A., Budde B.B. (2005)

Application of measurements of transepithelial electrical resistance of intestinal epithelial cell monolayers to evaluate probiotic activity. *Appl Environ Microbiol.* 71, pp. 7528-7530

Kolawole J.A., Mustapha A., Abudu-Aguye I., Ochekpe N. (2000) Mefloquine pharmacokinetics in healthy subjects and in peptic ulcer patients after cimetidine administration. *Eur J Drug Metab Pharmacokinet.* 25, pp. 165-170

Kosoglou T., Salfi M., Lim J.M., Batra V.K., Cayen M.N., Affrime M.B. (2000) Evaluation of the pharmacokinetics and electrocardiographic pharmacodynamics of loratadine with concomitant administration of ketoconazole or cimetidine. *Br J Clin Pharmacol.* 50, pp. 581-589

Lazzaroni M, Imbimbo B.P., Bargiggia S., Sangaletti O., Dal Bo L., Broccali G., Bianchi Porro G.. (1993) Effects of magnesium-aluminum hydroxide antacid on absorption of rufloxacin. *Antimicrob. Agents Chemother.* 37, pp. 2212-2216

Narumi S., Kumiko T., Akihiro M., Kenji A., Hiroshi T., Koji F. (2006) Effect of benzo[a]pyrene on P-glycoprotein-mediated transport in Caco-2 cell monolayer. *Toxicology* 23, pp. 156-165

Neu H.C. (1992) Quinolone antimicrobial agents. *Annu. Rev. Med.* 43, pp. 465-486

Pan B.H., Dutt A., Nelson A. (1994) Enhanced transepithelial flux of cimetidine by Madin-Darby Canine Kidney cells overexpressing human P-glycoprotein. *J. Pharmacol. Exp. Ther.*, 270, pp. 1-7

Pedersen K.E., Chrstiansen B.D., Klitgaard N.A., Nielsen-Kudsk, F. (1983) Effect of quinidine on dioxin bioavailability. *Eur. J. Clin. Pharmacol.* 24, pp. 41-47

Rubas W., Jezuk N., Grass G.M. (1993) Comparison of the permeability characteristics of a human colonic epithelial (Caco-2) cell line to colon of rabbit, monkey, and dog intestine and human drug absorption. *Pharm. Res.* 10, pp.113-118

Schinked, A.H., Mayer U., Wagenaar E., Mol C.A., van Deemter L. Smit J.J, van der Valk M.A., Voordouw A.C., Spits H., van Tellingen O., Zijlmans J.M., Fibbe W.E., Borst P. (1997) Normal viability and altered pharmacokinetics in mice lacking mdr1-type (drug-transporting) P-glycoproteins. *Proc. Nat., Acad. Sci.* 94, pp. 4028-4033

Shimada J., Shiba K., Oguma T., Miwa H., Yoshimura Y., Nishikawa T., Okabayashi Y., Kitagawa T., Yamamoto S. (1992) Effect of antacid on absorption of the quinolone lomefloxacin. *Antamicrob. Agents Chemother.* 36, pp. 1219-1224

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, pp. 3-12

Weidekamm E., Portmann R., Suter K., Partos C., Dell D., Lucker P.W. (1987) Single- and multiple-dose pharmacokinetics of fleroxacin, a trifluorinate quinolone, in humans. *Antimicrob. Agents Chemother.* 31, pp. 1909-1914

Wise R. (1984) Norfloxacin, a review of pharmacology and tissue penetration. *J. Antimicrobiol. Chemother.* 13, pp. 59-64

Guidance for Industry. Waiver of in vivo Bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system. CDER, FDA, August, 2000