Laboratory of Biopharmaceutics and Pharmacokinetics, Faculty of Pharmaceutical Sciences, Hiroshima International University, Hiroshima, Japan

Fate of quinidine, a P-glycoprotein substrate, in the gastrointestinal tract after oral administration in rats

N. Mori, T. Yokooji, T. Murakami

Received September 10, 2007, accepted November 2, 2007

Prof. Dr. Teruo Murakami, Laboratory of Biopharmaceutics and Pharmacokinetics, Faculty of Pharmaceutical Sciences, Hiroshima international University, 5–1–1 Hiro-koshingai, Kure, Hiroshima 737–0112, Japan
t-muraka@ps.hirokoku-u.ac.jp

Pharmazie 63: 241–244 (2008) *doi: 10.1691/ph.2008.7771*

P-Glycoprotein (P-gp), an ATP-dependent efflux transporter, is expressed in brush-border membranes in the intestines of humans and rodents. In this study, the fate of quinidine, a P-gp substrate, in the gastrointestinal tract after oral administration was examined in conscious rats. The animals received quinidine (3 mg/ml/kg) or FITC-dextran of molecular weight of 10,000 (FD-10S, 5 mg/ml/kg, a poorly absorbable compound) orally, and the remaining amount of the compound in the upper gastrointestinal tract was measured at designated time intervals. As a control, FD-10S was distributed almost evenly throughout the gastrointestinal tract at 30 min, and most of FD-10S was accumulated in the distal small intestine at 60 min after administration. In contrast, most of the orally administered quinidine disappeared at the proximal intestine, and only small amounts reached the distal region. Also, the gastrointestinal transit of FD-10S was markedly slowed by stopping or inhibiting the bile flow, indicating that bile flow significantly affects the transit of drugs in the gastrointestinal tract. In conclusion, this study has demonstrated that compounds with high solubility and high permeability, such as quinidine, can be absorbed rapidly at the proximal intestine, escaping the barrier function of P-gp, because P-gp is mostly expressed in the distal intestine.

1. Introduction

The gastrointestinal tract is exposed to various harmful exogenous compounds. However, it is equipped with various metabolizing enzymes and efflux transporters which play a role as a host defense/detoxification system. As ATP-dependent efflux transporters, P-glycoprotein (P-gp), breast cancer resistance protein (BCRP) and members of multidrug resistance-associated proteins (MRPs) are normally expressed in the intestines of humans and rodents. These efflux transporters are mainly localized on the apical brush-border membranes, playing a role in limiting influx and facilitating efflux to prevent the intracellular accumulation of their own substrate compounds. In contrast, some transporters, such as MRP3, are localized on the basolateral membrane of enterocytes, and increase the transport of their substrate compounds to the systemic circulation from the intracellular compartment (Yokooji et al. 2007a, b). P-gp transports a variety of structurally and pharmacologically unrelated neutral and positively charged hydrophobic compounds, while BCRP and MRPs transport relatively hydrophilic compounds including various conjugates of endogenous and exogenous substances (Suzuki and Sugiyama 2002; Chan et al. 2004; Doyle and Ross 2003; Takano et al. 2006). P-gp is considered the mainstay among various efflux transporters in considering oral bioavailability of drugs, since P-gp recognizes many clinically important hydrophobic compounds, including anticancer agents, calcium channel blockers, immunosuppressive agents, steroids, β-blockers, cardiac glycosides, and so on (Takano et al. 2006). Therefore, P-gp can limit the influx of orally administered substrate compounds in the intestine and facilitate the efflux of substrate compounds that circulate in the central blood flow to the intestinal lumen, and cause P-gp-mediated drug interaction in the intestine. Thus, under ordinary circumstances, a sufficient oral absorption (oral bioavailability) of the P-gp substrate compounds is unlikely. However, in clinical practice, there are many useful P-gp substrate drugs that are applied to the body through oral administration, such as diltiazem, nicardipine, nifedipine, verapamil, cyclosporin A, tacrolimus, cortisol, dexamethasone, tamoxifen, digoxin, digitoxin, α-methyldigoxin, β-acetyldigoxin, erythromycin, quinidine, ketoconazole, itraconazole, etc. In order to resolve the problem of why these P-gp substrate compounds could be applied orally, we examined the fate of quinidine, a P-gp substrate, in the gastrointestinal tract after oral administration in conscious rats. In this study, we chose quinidine as a model compound based on the fact that the membrane transport of quinidine across the intestine and the blood-brain barrier has been known to be mediated by P-gp (Kusuhara et al. 1997; Emi et al. 1998; Adachi et al. 2003). It has been categorized as a class 1 compound with high solubility and high permeability in

the Biopharmaceutical Classfication System (BCS) defined by the FDA (Amidon et al. 1995; Wu and Benet 2004), and the oral bioavailability of quinidine is more than 80% in humans (Greenblatt et al. 1977).

2. Investigations, and results

2.1. Fate of FD-10S in the intestine after oral administration

FD-10S was used to evaluate the gastrointestinal transit of intestinal fluid, since this hydrophilic macromolecule compound is not absorbed in the intestine. In the experiment, the remaining amounts of FD-10S in the intestine were measured 15 min, 30 min and 60 min after oral administration (Fig. 1). The total recovery rate of FD-10S from the whole intestinal lumen was almost 100% at all three time points examined. At 15 min after administration, FD-10S remained mostly at the proximal region, including the stomach; at 30 min, FD-10S was distributed evenly throughout the gastrointestinal tract; and at 60 min, most of FD-10S was accumulated in the distal small intestine.

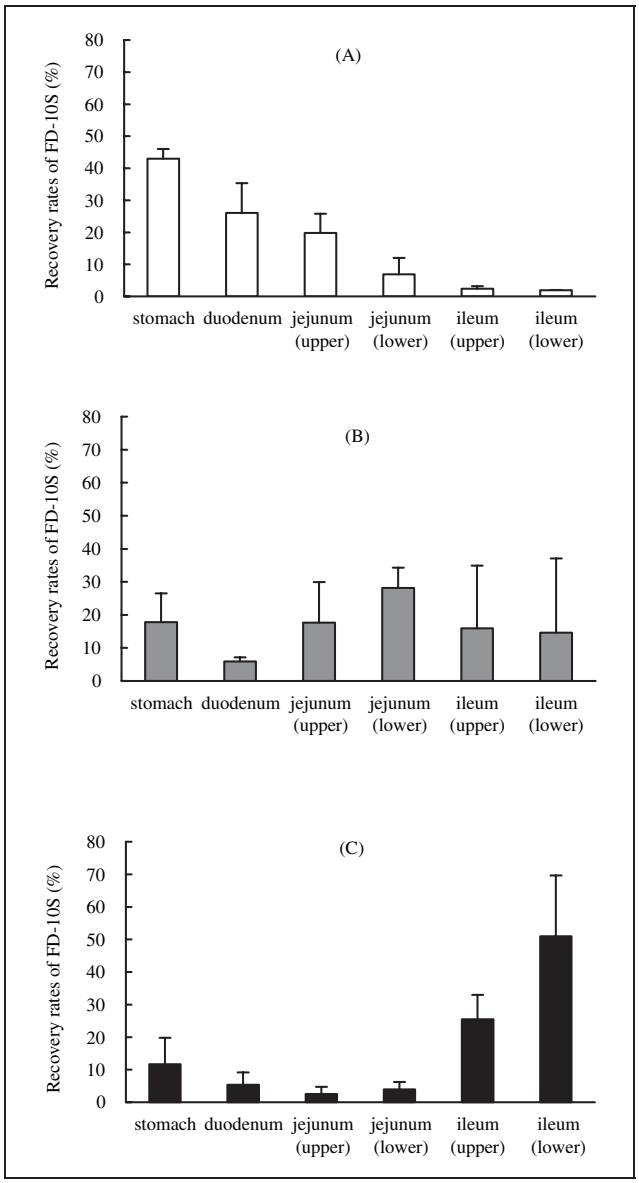

Fig. 1: Time profile for the remaining amounts of FD-10S in the intestine after oral administration in rats. FD-10S was administered orally at a dose of 5 mg/ml/kg. Animals were sacrificed at 15 min (A), 30 min (B) and 60 min (C) after the administration. Each value represents mean ± S.D. of 3 trials

2.2. Fate of quinidine in the intestine after oral administration

After oral administration, quinidine remained mostly at the proximal intestine, regardless of the elapsed time, and only a small amount of quinidine reached the distal intestine (Fig. 2). The total recovery amounts of quinidine from the intestine decreased as the time passed: 67% at 15 min, 41% at 30 min, and 29% at 60 min after oral administration. These results suggest that quinidine discharged from the stomach was rapidly absorbed from the proximal intestine before reaching the distal intestine.

2.3. Effect of bile flow on the fate of FD-10S in the intestine after oral administration

The effect of bile flow on the fate of FD-10S was examined in two different cholestesis models: bile-duct ligated rats and α-naphtylisothiocyanate (ANIT)-treated rats. In the bile-duct ligated model, as shown in Fig. 3, the gastric emptying rate of FD-10S was markedly lower, and the

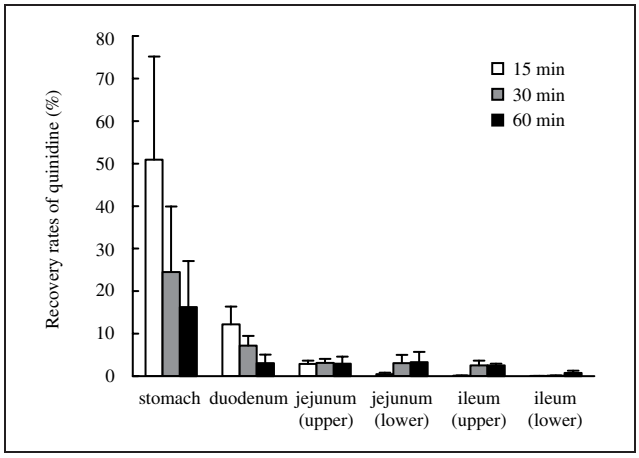

Fig. 2: Time profile for the remaining amounts of quinidine in the intestine after oral administration in rats. Quinidine was administered orally at a dose of 3 mg/ml/kg. Animals were sacrificed at 15 min (□), 30 min (▨), and 60 min (■) after the administration. Each value represents mean ± S.D. of 3 trials

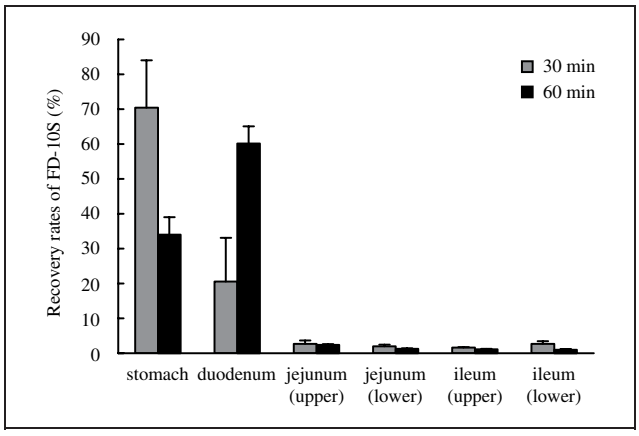

Fig. 3: Time profile for the remaining amounts of FD-10S in the intestine after oral administration in the bile-duct ligated rats. At 2 h after the bile duct ligation, FD-10S was administered at a dose of 5 mg/ml/kg. Animals were sacrificed at 30 min (▨) or 60 min (■) after FD-10S administration. Each value represents mean ± S.D. of 3 trials

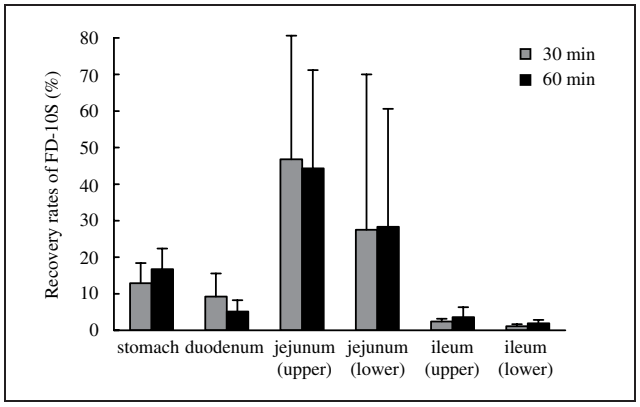

Fig. 4: Time profile for the remaining amounts of FD-10S in the intestine after oral administration in the ANIT-treated rats. Animals were treated with ANIT one day prior to FD-10S administration. FD-10S was administered at a dose of 5 mg/ml/kg. Animals were sacrificed 30 min (□) or 60 min (■) after FD-10S administration. Each value represents mean ± S.D. of 3 trials

recovery rate of FD-10S from the stomach at 30 min and 60 min after oral administration was higher than that in normal rats: 70% vs. 18%, and 34% vs. 12%, respectively. The discharged FD-10S from the stomach was mostly retained at the duodenum. In the ANIT-treated rats, the gastric emptying rate of FD-10S was the same as that in normal rats; however, the intestinal transit of FD-10S was remarkably lower. In this ANIT-treated model, FD-10S mostly accumulated in the central region of the small intestine and did not reach the distal intestine even at 60 min after oral administration (Fig. 4).

3. Discussion

In human intestine, P-gp is abundantly expressed in the distal intestine, such as the ileum and colon, while the expression gradually decreases towards the jejunum, duodenum and stomach (Ho et al. 2003; Mouly and Paine 2003). Such a regional difference in P-gp expression and/or function is also observed in rodent intestine (Chianale et al. 1995; Fricker et al. 1996; Yumoto et al. 1999). In the report of Yumoto et al. (1999), the P-gp function was evaluated by determining the P-gp-mediated efflux transport of rhodamine 123, a well-known typical P-gp substrate, from serosal to mucosal surfaces in the everted rat intestine. They found several-fold higher P-gp function at the distal intestine (the lower 2/5 portions of the small intestine) than that at the proximal intestine. Such site specific expression and function of efflux transporters, especially P-gp, in the intestine would affect the oral bioavailability of many P-gp substrate drugs, depending on the absorption sites of these orally administered drugs. For example, it is reported that the oral bioavailability of digoxin, cyclosporin A and tacrolimus correlates well with the amounts of P-gp expressed in the small intestine (Lown et al. 1997; Greiner et al. 1999; Masuda et al. 2000). In the present study, quinidine was used as a model typical P-gp substrate. Adachi et al. (2003) determined the permeability-surface area (PS) product for 12 well-known P-gp substrate drugs by an *in situ* intestinal perfusion technique in mdr1a/1b (−/−) and normal mice in order to evaluate the extent to which the intestinal absorption is affected by P-gp. They found that the PS product of quinidine in mdr1a/1b (−/−) mice was much higher than that in normal mice. Mizuno et al. (2003) reported a higher Kp (brain-plasma partition coefficient) value ratio of quinidine in mdr1a/1b (−/−) than that in mdr1a/1b (+/+) mice, indicating a higher contribution of P-gp in the brain distribution of quinidine.

After oral administration, quinidine disappeared rapidly at the proximal intestine and did not reach the distal intestine (Fig. 2), though the intestinal fluids, as evaluated by the intestinal distribution of FD-10S, ran through the whole small intestine (Fig. 1). The rapid oral absorption of quinidine has also been reported in humans, where the mean first-order absorption half-life was 38.0 ± 5.5 min. (Greenblatt et al. 1977). In these cases, the amount of quinidine that interacted with P-gp was considerably low, since P-gp had a much lower expression in the proximal intestine (Chianale et al. 1995; Fricker et al. 1996; Yumoto et al. 1999). Thus, most of the administered quinidine was absorbed to the systemic system through the proximal intestine. In the present study, we also examined the effect of bile flow on the gastrointestinal transit of orally administered drugs, since bile juice contains various bile acids, the endogenous surface active agents that affect the interfacial tension between the luminal fluids (intestinal contents) and intestinal membrane. Using FD-10S (not absorbable from the intestine) as a marker, the effect of the bile flow on the transit pattern of the orally administered drugs in the intestine was investigated. As shown in Fig. 3 and Fig. 4, the gastrointestinal transit of FD-10S was markedly reduced by stopping (Fig. 3) or inhibiting (Fig. 4) the bile flow, indicating that bile flow plays an important role in the gastrointestinal transit of food or drugs. In the bile-duct ligated rats, most of the administered FD-10S still remained in the stomach and duodenum after 60 min, while in the ANIT-treated rats, due to the fact that bile flow was not completely suppressed (Kossor et al. 1993a, b; Jean and Roth 1995), FD-10S was transferred to the central region of the intestine after 30 min to 60 min. The difference in the gastrointestinal transit of FD-10S between the bile duct ligated and ANIT-treated rats also demonstrates the importance of bile flow in the transit of drugs.

Amidon et al. devised a BCS to classify drugs into 4 classes according to their solubility and permeability (Amidon et al. 1995; Wu and Benet 2005). In the BCS, some P-gp substrates such as quinidine, nifedipine and verapamil are classified into class 1 (high solubility and high permeability compounds). On the other hand, many P-gp substrates such as cyclosporine, digoxin, erythromycin, indinavir, nelfinavir, saquinavir, tacrolimus and terfenadine are classified into class 2 (low solubility and high permeability compounds). If the solubility of the compound is low, some portion of the compound administered orally will reach the distal intestine, which may cause P-gp-mediated drug interaction. In fact, the oral bioavailability of some class 2 compounds such as digoxin, cyclosporin A and tacrolimus have been reported to be affected by the amounts of P-gp expressed in the small intestine (Lown et al. 1997; Greiner et al. 1999; Masuda et al. 2000). Thus, the participation of P-gp in the intestinal absorption of a compound could depend on the solubility and permeability of that compound. The compounds with both high solubility and high permeability, such as quinidine, would be absorbed rapidly at the proximal intestine, and would escape, at least partly, the barrier function of P-gp, because P-gp is mostly expressed in the distal intestine. In conclusion, increasing the membrane permeability and solubility is important to the absorption of a compound in the intestine, not only to escape the absorption barrier made by the presence of P-gp, but also to avoid the intestinal first-pass metabolism, at least partially, due to the higher absorption rate of the compound in the proximal area.

4. Experimental

4.1. Chemicals and reagents

Quinidine and 1-naphthyl isothiocyanate (ANIT) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Fluoresceine isothiocyanate-dextran with molecular weight of 10,000 (FD-10S) was obtained from Sigma-Aldrich Japan K. K. (Tokyo, Japan). All other chemicals and reagents were of the highest purity available.

4.2. Animal studies

Male Sprague-Dawley (SD) rats weighing about 250 to 350 g were purchased from Japan SLC, Inc. (Shizuoka, Japan). Rats were fed a standard laboratory diet (CE-2, Clea Japan, INC., Tokyo, Japan) and water for more than 1 week prior to the experiments. All animal experiments were performed in accordance with the guidelines for proper conduct of animal experiments from Science Council of Japan that is open to the public on internet. Rats were fasted overnight (except for ANIT treatment) with free access of water prior to the oral administration of quinidine or FD-10S.
Cholestesis model rats were made by the methods of bile duct ligated and ANIT treatment. In bile duct ligated method, the extrahepatic bile duct of the fasted rats was ligated under light anesthesia (with ethyl ether), then the abdominal operation site was stitched up, and the animals were used 2 h later for the intestinal transition experiments. In the ANIT treatment method, ANIT was dissolved in olive oil at a concentration of 50 mg/ml. Following the method of the previous report (Kossor et al. 1993a, b; Jean and Roth 1995), the ANIT solution was administered orally to non-fasted rats by stomach intubation at a dose of 100 mg/kg (dosing volume of 2 mL/kg). Subsequently, the animals were fasted for 24 h and used for the intestinal distribution experiments. Quinidine and FD-10S were dissolved in distilled water at the concentrations of 3 mg/ml and 5 mg/ml, respectively. For obtaining a quinidine solution, 10 µl of 5 M HCl was added to each mL of quinidine suspension to aid the solubility. Drug (quinidine & FD-10S) solutions were administered orally by stomach intubation at a dose of 3 mg/kg and 5 mg/kg (dosing volume of 1 ml/kg), respectively. At 1 min prior to the designated time (15 min, 30 min, or 60 min after the administration), rats were lightly anesthetized with ethyl ether, and sacrificed by a heart puncture of an excess amount of sodium pentobarbital. Subsequently, the abdomen was opened and the whole small intestine was exposed. Ligation was made at the cardiac opening, the pyloric part, and the duodenum (15 cm down from the pyloric part), and the remaining small intestine was further divided and ligated into four parts of equal length (approximately 20 cm each). The resulting gastrointestinal loops were isolated, and each loop was weighed, added with 9-fold volumes of distilled water, and homogenized with a tissue homogenizer (21,000 rpm, 2 min). The homogenates were stored at −30 °C until analyzed.

4.3. Analysis

The concentration of quinidine in the tissue homogenate was determined by HPLC (Nishiura et al. 1986). A 1 ml of 0.1 M NaOH was added to 0.2 ml of tissue homogenate, and the suspension was extracted with 5 ml of ethylacetate. The ethylacetate layer (4 ml) was evaporated under reduced pressure and the residue was dissolved with 0.1 ml of HPLC mobile phase (acetonitrile/methanol/50 mM, pH 3.0 phosphate buffer = 4/2/4, v/v). For HPLC, a Lichrospher 100 RP-18(e) column (Cica-Merk, Tokyo, Japan) and a fluorescence detector operating at wavelengths of 310 nm (excitation) and 380 nm (emission) were used. For FD-10S determination, the homogenate was centrifuged at 10,000 rpm for 5 min, and the supernatant (50 µL) was properly diluted with distilled water. The fluorescence intensity was measured at wavelengths of 496 nm for excitation and 516 nm for emission.

This research paper was presented during the 6th Conference on Retrometabolism Based Drug Design and Targeting, June 3–6, 2007, Göd, Hungary.

References

Adachi Y, Suzuki H, Sugiyama Y (2003) Quantitative evaluation of the function of small intestinal P-glycoprotein: comparative studies between in situ and in vitro. Pharm Res 20: 1163–1169.

Amidon GL, Lennernas H, Shah VP, Crison JR (1995) A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm Res 12: 413–420.

Chan LM, Lowes S, Hirst BH (2004) The ABCs of drug transport in intestine and liver: efflux proteins limiting drug absorption and bioavailability. Eur J Pharm Sci 21: 25–51.

Chianale J, Vollrath V, Wielandt AM, Miranda S, Gonzalez R, Fresno AM, Quintana C, Gonzalez S, Andrade L, Guzman S (1995) Differences between nuclear run-off and mRNA levels for multidrug resistance gene expression in the cephalocaudal axis of the mouse intestine. Biochim Biophys Acta 1264: 369–376.

Doyle LA, Ross DD (2003) Multidrug resistance mediated by the breast cancer resistance protein BCRP (ABCG2). Oncogene 22: 7340–7358.

Emi Y, Tsunashima D, Ogawara K, Higaki K, Kimura T (1998) Role of P-glycoprotein as a secretory mechanism in quinidine absorption from rat small intestine. J Pharm Sci 87: 295–299.

Fricker G, Drewe J, Huwyler J, Gutmann H, Beglinger C (1996) Relevance of p-glycoprotein for the enteral absorption of cyclosporin A: in vitro-in vivo correlation. Br J Pharmacol 118: 1841–1847.

Greenblatt DJ, Pfeifer HJ, Ochs HR, Franke K, MacLaughlin DS, Smith TW, Koch-Weser J (1977) Pharmacokinetics of quinidine in humans after intravenous, intramuscular and oral administration. J Pharmacol Exp Ther 202: 365–378.

Greiner B, Eichelbaum M, Fritz P, Kreichgauer HP, von Richter O, Zundler J, Kroemer HK (1999) The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. J Clin Invest 104: 147–153.

Ho GT, Moodie FM, Satsangi J (2003) Multidrug resistance 1 gene (P-glycoprotein 170): an important determinant in gastrointestinal disease? Gut 52: 759–766.

Jean PA, Roth RA (1995) 1-naphthylisothiocyanate-induced elevation of biliary glutathione. Biochem Pharmacol 50: 1469–1474.

Kossor DC, Handler JA, Dulik DM, Meunier PC, Leonard TB, Goldstein RS (1993a) Cholestatic potentials of alpha-naphthylisothiocyanate (ANIT) and beta-naphthylisothiocyanate (BNIT) in the isolated perfused rat liver. Biochem Pharmacol 46: 2061–2066.

Kossor DC, Meunier PC, Handler JA, Sozio RS, Goldstein RS (1993b) Temporal relationship of changes in hepatobiliary function and morphology in rats following alpha-naphthylisothiocyanate (ANIT) administration. Toxicol Appl Pharmacol 119: 108–114.

Kusuhara H, Suzuki H, Terasaki T, Kakee A, Lemaire M, Sugiyama Y (1997) P-Glycoprotein mediates the efflux of quinidine across the blood-brain barrier. J Pharmacol Exp Ther 283: 574–580.

Lown KS, Mayo RR, Leichtman AB, Hsiao HL, Turgeon DK, Schmiedlin-Ren P, Brown MB, Guo W, Rossi SJ, Benet LZ, Watkins PB (1997) Role of intestinal P-glycoprotein (mdr1) in interpatient variation in the oral bioavailability of cyclosporine. Clin Pharmacol Ther 62: 248–260.

Masuda S, Uemoto S, Hashida T, Inomata Y, Tanaka K, Inui K (2000) Effect of intestinal P-glycoprotein on daily tacrolimus trough level in a living-donor small bowel recipient. Clin Pharmacol Ther 68: 98–103.

Mizuno N, Niwa T, Yotsumoto Y, Sugiyama Y (2003) Impact of drug transporter studies on drug discovery and development. Pharmacol Rev 55: 425–461.

Mouly S, Paine MF (2003) P-glycoprotein increases from proximal to distal regions of human small intestine. Pharm Res 20: 1595–1599.

Nishiura A, Higashi J, Murakami T, Higashi Y, Yata N (1986) A possible contribution of phospholipids in tissue distribution of quinidine in rats. J Pharmacobiodyn 9: 819–828.

Suzuki H, Sugiyama Y (2002) Single nucleotide polymorphisms in multidrug resistance associated protein 2 (MRP2/ABCC2): its impact on drug disposition. Adv Drug Deliv Rev 54: 1311–1331.

Takano M, Yumoto R, Murakami T (2006) Expression and function of efflux drug transporters in the intestine. Pharmacol Ther 109: 137–1361.

Wu CY, Benet LZ (2005) Predicting drug disposition via application of BCS: transport/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system. Pharm Res 22: 11–23.

Yokooji T, Murakami T, Yumoto R, Nagai J, Takano M (2007a) Site-specific bidirectional efflux of 2,4-dinitrophenyl-S-glutathione, a substrate of multidrug resistance-associated proteins, in rat intestine and Caco-2 cells. J Pharm Pharmacol 59: 513–520.

Yokooji T, Murakami T, Yumoto R, Nagai J, Takano M (2007b) Role of intestinal efflux transporters in the intestinal absorption of methotrexate in rats. J Pharm Pharmacol 59: 1263–1270.

Yumoto R, Murakami T, Nakamoto Y, Hasegawa R, Nagai J, Takano M (1999) Transport of rhodamine 123, a P-glycoprotein substrate, across rat intestine and Caco-2 cell monolayers in the presence of cytochrome P-450 3A-related compounds. J Pharmacol Exp Ther 289: 149–155.