kidney slices, respectively) (Nozaki et al., 2004; this study). Secondly, the NSAIDs, except for ketoprofen, inhibited the unknown transporter more potently in human kidney slices than in rat kidney slices. In particular, the K_i value of salicylate determined in human kidney slices was smaller than that for OAT3 (Tables 1 and 2). These NSAIDs may be more potent inhibitors of this unknown transporter than OAT3. As suggested in rodents, RFC-1 is a candidate transporter. In addition, recently, proton-coupled folate transporter/heme carrier protein 1 (PCFT/HCP1) was also identified as a novel MTX transporter, which is also expressed in the kidney, at least, at the mRNA level (Qiu et al., 2006). This transporter may be another candidate transporter. Further studies are required to elucidate their importance.

Human kidney slice studies also suggested that diclofenac, ketoprofen, and naproxen do not inhibit the uptake of MTX at clinical concentrations, although they have caused drug-drug interactions with MTX in clinical situations (Thyss et al., 1986; Ng et al., 1987; Tracy et al., 1992; Davies and Anderson, 1997a). Because renal tubular secretion involves excretion into the lumen through the BBM of the proximal tubules, inhibition of apical efflux transporters can also serve as an alternative interaction site. Therefore, the effect of NSAIDs was examined for the ATP binding cassette transporters, such as MRP2, BCRP, and MRP4, which accept MTX as a substrate. ATP-dependent transport of MTX was observed in BCRP-, MRP2-, and MRP4-expressing vesicles (Fig. 4, A–C). The K_m values of MTX for BCRP, MRP2, and MRP4 were consistent with previously reported values (Bakos et al., 2000; Mitomo et al., 2003; Volk and Schneider, 2003). The

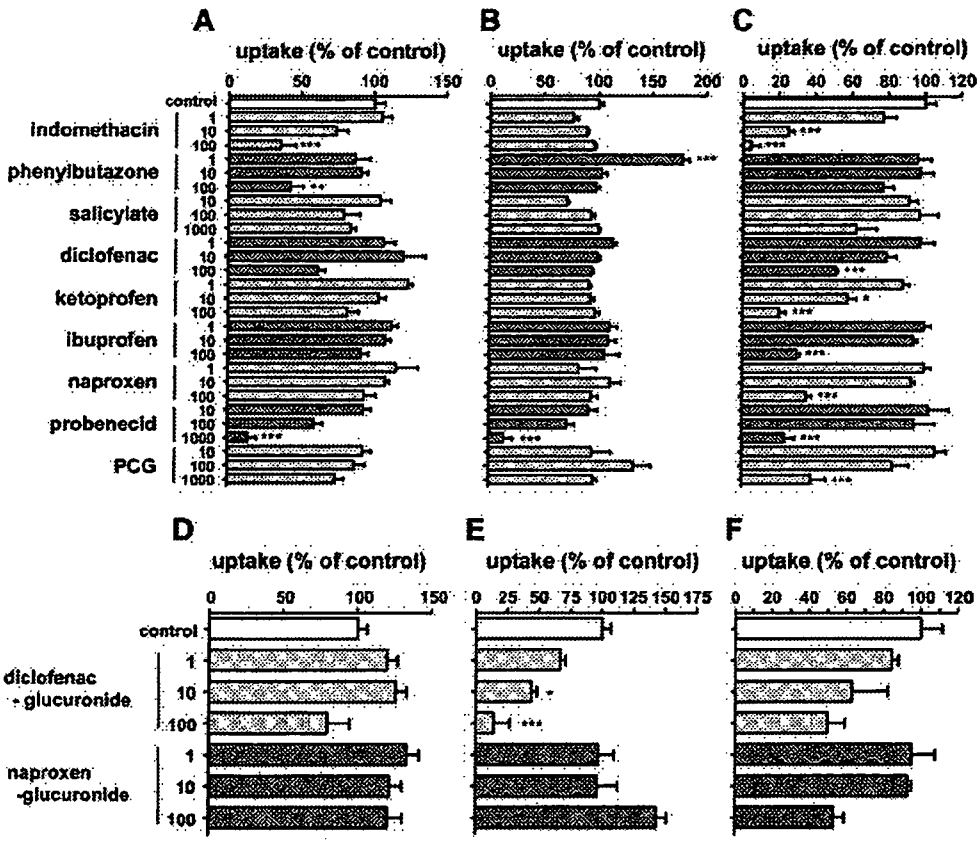

Fig. 5. Inhibitory effect of NSAIDs and other drugs on BCRP-, MRP2-, and MRP4-mediated transport of MTX. The uptake of MTX (0.1 μM) by membrane vesicles prepared from HEK293 cells infected BCRP, MRP2, and MRP4 adenoviruses was measured for 5 min at 37°C in the presence or absence of inhibitors (A–C, respectively). Values are given by subtracting the uptake clearance in the presence of AMP from that in the presence of ATP and are shown as a percentage of the uptake in the absence of inhibitors. Inhibitory effect of diclofenac- and naproxen-glucuronides on the BCRP-, MRP2-, and MRP4-mediated transport of MTX (D–F, respectively). Each value represents the mean ± S.E. (n = 3). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ statistically different from control.

TABLE 3

Quantitative evaluation of drug-drug interactions between MTX and NSAIDs via MRP4

Inhibitory effect of NSAIDs on MRP4-mediated transport of MTX was examined, and K_i values were determined by nonlinear regression analysis. All K_i values represent the mean ± S.D. Plasma unbound concentrations of the inhibitors (I_u) were calculated from the total plasma concentrations and unbound fractions.

Inhibitors	Clinical Concentration		K_i	R Value
	Total	I_u		
		μM		
Salicylate	1100–2200[a]	55–440	218	0.33–0.80
Diclofenac	3.6[b]	<0.018	>100	1.0
Indomethacin	0.84–84[a]	0.084–8.4	2.95	0.26–0.97
Ibuprofen	48.5[a]	<0.485	73.3	1.0
Ketoprofen	12[a]	0.0096	23.3	1.0
Naproxen	>217[a]	0.651	75.3	0.99
Phenylbutazone	162–786[a]	6.3–19.0	354	0.95–0.98

[a] Takeda et al. (2002).
[b] Riess et al. (1978).

effect of NSAIDs, probenecid, and PCG on the BCRP-, MRP2-, and MRP4-mediated transport of MTX was examined (Fig. 5, A–C, respectively). NSAIDs showed only a weak or minimal effect on MRP2 (Fig. 5B), which is consistent with a previous report (Horikawa et al., 2002). Because NSAIDs are mainly excreted into the urine as the glucuronide-conjugated form (Davies and Anderson, 1997a,b), we evaluated the inhibitory effect of diclofenac and naproxen glucuronide, which were prepared biosynthetically in vitro, on MRP2-mediated transport of MTX (Fig. 5E). Diclofenac glucuronide significantly inhibited the MRP2-mediated transport of MTX in a concentration-dependent manner (Fig. 5E). Therefore, this drug-drug interaction may involve inhibition of MRP2 by the glucuronide conjugate, but not the parent compound, although the clinical relevance of this inhibition remains unknown. BCRP-mediated transport of MTX was significantly inhibited by 100 μM indomethacin and phenylbutazone and 1000 μM probenecid (Fig. 5A). However, such inhibition was not clinically relevant considering their unbound plasma concentrations in clinical situations. MRP4 is more susceptible to NSAIDs compared with BCRP and MRP2 (Fig. 5C), which agrees with very recently published results (El-Sheikh et al., 2007). Salicylate, indomethacin, ibuprofen, ketoprofen, naproxen, and phenylbutazone inhibited MRP4-mediated transport of MTX in a concentration-dependent manner, and the K_i values of these NSAIDs for MRP4 were generally comparable with previous results with some exceptions. Salicylate exhibited no inhibition of MRP4-mediated MTX transport at a concentration of 100 μM (Fig. 5). Addition of experimental points at higher concentrations gave K_i values of 218 μM, although the K_i value was 7-fold smaller than the previously reported values for some unknown reason. Based on R values (Table 3), salicylate can be expected to inhibit MRP4-mediated transport at clinical doses, and indomethacin also has a potential to inhibit MRP4 at high clinical concentrations. Because several NSAIDs are substrates of OAT1 (Apiwattanakul et al., 1999), it is possible that NSAIDs, concentrated in the renal tubular cells by basolateral organic anion transporter(s), may exhibit a greater inhibition than expected from the plasma unbound concentrations. It must be kept in mind that the impact of the inhibition of MRP4 by salicylate and/or indomethacin on the renal elimination of MTX totally depends on the contribution of MRP4 to the net efflux across the BBM. It is required to evaluate the contribution of apical efflux transporters in the future for more reliable prediction.

In conclusion, the present study suggests that drug-drug interactions between MTX and salicylate, indomethacin, phenylbutazone, and probenecid involve inhibition of the uptake mediated by OAT3 and other unknown transporters. The transport studies using human kidney slices demonstrated an interspecies difference in the inhibition potencies of NSAIDs, indicating the importance of using human materials for the quantitative prediction of drug-drug interactions. As far as MRP4 is concerned, salicylate and indomethacin were predicted to have a significant effect in clinical situations. In addition to the parent compounds, drug-drug interactions may involve the inhibition of apical ATP-binding cassette transporters (MRP2 and MRP4) by glucuronide conjugates of NSAIDs.

References

Aherne GW, Piall E, Marks V, Mould G, and White WF (1978) Prolongation and enhancement of serum methotrexate concentrations by probenecid. *Br Med J* **1**:1097–1099.

Apiwattanakul N, Sekine T, Chairoungdua A, Kanai Y, Nakajima N, Sophasan S, and Endou H (1999) Transport properties of nonsteroidal anti-inflammatory drugs by organic anion transporter 1 expressed in *Xenopus laevis* oocytes. *Mol Pharmacol* **55**:847–854.

Bakos E, Evers R, Sinko E, Varadi A, Borst P, and Sarkadi B (2000) Interactions of the human multidrug resistance proteins MRP1 and MRP2 with organic anions. *Mol Pharmacol* **57**:760–768.

Breedveld P, Zelcer N, Pluim D, Sonmezer O, Tibben MM, Beijnen JH, Schinkel AH, van Tellingen O, Borst P, and Schellens JH (2004) Mechanism of the pharmacokinetic interaction between methotrexate and benzimidazoles: potential role for breast cancer resistance protein in clinical drug-drug interactions. *Cancer Res* **64**:5804–5811.

Brunton LL, Lazo JS, and Parker KL eds (2006) *Goodman & Gilaman's The Pharmacological Basis of Therapeutics*, McGraw Hill, New York, NY.

Cha SH, Sekine T, Fukushima JI, Kanai Y, Kobayashi Y, Goya T, and Endou H (2001) Identification and characterization of human organic anion transporter 3 expressing predominantly in the kidney. *Mol Pharmacol* **59**:1277–1286.

Dalle JH, Auvrignon A, Vassal G, and Leverger G (2002) Interaction between methotrexate and ciprofloxacin. *J Pediatr Hematol Oncol* **24**:321–322.

Davies NM and Anderson KE (1997a) Clinical pharmacokinetics of diclofenac: therapeutic insights and pitfalls. *Clin Pharmacokinet* **33**:184–213.

Davies NM and Anderson KE (1997b) Clinical pharmacokinetics of naproxen. *Clin Pharmacokinet* **32**:268–293.

Ellison NM and Servi RJ (1985) Acute renal failure and death following sequential intermediate-dose methotrexate and 5-FU: a possible adverse effect due to concomitant indomethacin administration. *Cancer Treat Rep* **69**:342–343.

El-Sheikh AA, van den Heuvel JJ, Koenderink JB, and Russel FG (2007) Interaction of nonsteroidal anti-inflammatory drugs with multidrug resistance protein (MRP) 2/ABCC2- and MRP4/ABCC4-mediated methotrexate transport. *J Pharmacol Exp Ther* **320**:229–235.

Fleck C, Hilger R, Jurkutat S, Karge E, Merkel U, Schimske A, and Schubert J (2002) Ex vivo stimulation of renal transport of the cytostatic drugs methotrexate, cisplatin, topotecan (Hycamtin) and raltitrexed (Tomudex) by dexamethasone, T3 and EGF in intact human and rat kidney tissue and in human renal cell carcinoma. *Urol Res* **30**:256–262.

Hasegawa M, Kusuhara H, Adachi M, Schuetz JD, Takeuchi K, and Sugiyama Y (2007) Multidrug resistance-associated protein 4 is involved in the urinary excretion of hydrochlorothiazide and furosemide. *J Am Soc Nephrol* **18**:37–45.

Horikawa M, Kato Y, Tyson CA, and Sugiyama Y (2002) The potential for an interaction between MRP2 (ABCC2) and various therapeutic agents: probenecid as a candidate inhibitor of the biliary excretion of irinotecan metabolites. *Drug Metab Pharmacokinet* **17**:23–33.

Hosoyamada M, Sekine T, Kanai Y, and Endou H (1999) Molecular cloning and functional expression of a multispecific organic anion transporter from human kidney. *Am J Physiol* **276**:F122–F128.

Hulot JS, Villard E, Maguy A, Morel V, Mir L, Tostivint I, William-Faltaos D, Fernandez C, Hatem S, Deray G, et al. (2005) A mutation in the drug transporter gene ABCC2 associated with impaired methotrexate elimination. *Pharmacogenet Genomics* **15**:277–285.

Imaoka T, Kusuhara H, Adachi M, Schuetz JD, Takeuchi K, and Sugiyama Y (2007) Functional involvement of multidrug resistance-associated protein 4 (MRP4/ABCC4) in the renal elimination of the antiviral drugs adefovir and tenofovir. *Mol Pharmacol* **71**:619–627.

Iwaki M, Bischer A, Nguyen AC, McDonagh AF, and Benet LZ (1995) Stereoselective disposition of naproxen glucuronide in the rat. *Drug Metab Dispos* **23**:1099–1103.

Li M, Anderson GD, and Wang J (2006) Drug-drug interactions involving membrane transporters in the human kidney. *Expert Opin Drug Metab Toxicol* **2**:505–532.

Liegler DG, Henderson ES, Hahn MA, and Oliverio VT (1969) The effect of organic acids on renal clearance of methotrexate in man. *Clin Pharmacol Ther* **10**:849–857.

Maïche AG (1986) Acute renal failure due to concomitant action of methotrexate and indomethacin. *Lancet* **1**:1390.

Mitomo H, Kato R, Ito A, Kasamatsu S, Ikegami Y, Kii I, Kudo A, Kobatake E, Sumino Y, and Ishikawa T (2003) A functional study on polymorphism of the ATP-binding cassette transporter ABCG2: critical role of arginine-482 in methotrexate transport. *Biochem J* **373**:767–774.

Ng HW, Macfarlane AW, Graham RM, and Verbov JL (1987) Near fatal drug interactions with methotrexate given for psoriasis. *Br Med J (Clin Res Ed)* **295**:752–753.

Nozaki Y, Kusuhara H, Endou H, and Sugiyama Y (2004) Quantitative evaluation of the drug-drug interactions between methotrexate and nonsteroidal anti-inflammatory drugs in the renal uptake process based on the contribution of organic anion transporters and reduced folate carrier. *J Pharmacol Exp Ther* **309**:226–234.

Nozaki Y, Kusuhara H, Kondo T, Hasegawa M, Shiroyanagi Y, Nakazawa H, Okano T, and Sugiyama Y (2007) Characterization of the uptake of organic anion transporter (OAT) 1 and OAT3 substrates by human kidney slices. *J Pharmacol Exp Ther* **321**:362–369.

Qiu A, Jansen M, Sakaris A, Min SH, Chattopadhyay S, Tsai E, Sandoval C, Zhao R, Akabas MH, and Goldman ID (2006) Identification of an intestinal folate transporter and the molecular basis for hereditary folate malabsorption. *Cell* **127**:917–928.

Riess W, Stierlin H, Degen P, Faigle JW, Gerardin A, Moppert J, Sallmann A, Schmid K, Schweizer A, Sulc M, et al. (1978) Pharmacokinetics and metabolism of the anti-inflammatory agent Voltaren. *Scand J Rheumatol Suppl* **17**:22–29.

Ronchera CL, Hernandez T, Peris JE, Torres F, Granero L, Jimenez NV, and Pla JM

(1993) Pharmacokinetic interaction between high-dose methotrexate and amoxycillin. *Ther Drug Monit* 15:375–379.

Russel FG, Masereeuw R, and van Aubel RA (2002) Molecular aspects of renal anionic drug transport. *Annu Rev Physiol* 64:563–594.

Sekine T, Watanabe N, Hosoyamada M, Kanai Y, and Endou H (1997) Expression cloning and characterization of a novel multispecific organic anion transporter. *J Biol Chem* 272:18526–18529.

Shitara Y, Sato H, and Sugiyama Y (2005) Evaluation of drug-drug interaction in the hepatobiliary and renal transport of drugs. *Annu Rev Pharmacol Toxicol* 45:689–723.

Tahara H, Kusuhara H, Endou H, Koepsell H, Imaoka T, Fuse E, and Sugiyama Y (2005) A species difference in the transport activities of H2 receptor antagonists by rat and human renal organic anion and cation transporters. *J Pharmacol Exp Ther* 315:337–345.

Takeda M, Khamdang S, Narikawa S, Kimura H, Hosoyamada M, Cha SH, Sekine T, and Endou H (2002) Characterization of methotrexate transport and its drug interactions with human organic anion transporters. *J Pharmacol Exp Ther* 302:666–671.

Thyss A, Milano G, Kubar J, Namer M, and Schneider M (1986) Clinical and pharmacokinetic evidence of a life-threatening interaction between methotrexate and ketoprofen. *Lancet* 1:256–258.

Titier K, Lagrange F, Pehourcq F, Moore N, and Molimard M (2002) Pharmacokinetic interaction between high-dose methotrexate and oxacillin. *Ther Drug Monit* 24:570–572.

Tracy TS, Krohn K, Jones DR, Bradley JD, Hall SD, and Brater DC (1992) The effects of a salicylate, ibuprofen, and naproxen on the disposition of methotrexate in patients with rheumatoid arthritis. *Eur J Clin Pharmacol* 42:121–125.

Volk EL and Schneider E (2003) Wild-type breast cancer resistance protein (BCRP/ABCG2) is a methotrexate polyglutamate transporter. *Cancer Res* 63:5538–5543.

Yamamoto K, Sawada Y, Matsushita Y, Moriwaki K, Bessho F, and Iga T (1997) Delayed elimination of methotrexate associated with piperacillin administration. *Ann Pharmacother* 31:1261–1262.

Yamaoka K, Tanigawara Y, Nakagawa T, and Uno T (1981) A pharmacokinetic analysis program (multi) for microcomputer. *J Pharmacobiodyn* 4:879–885.

Address correspondence to: Dr. Hiroyuki Kusuhara, Department of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyoku-Tokyo 13-0033, Japan. E-mail: kusuhara@mol.f.u-tokyo.ac.jp

0090-9556/08/3604-1–7$20.00
DRUG METABOLISM AND DISPOSITION
Copyright © 2008 by The American Society for Pharmacology and Experimental Therapeutics
DMD 36:1–7, 2008

Vol. 36, No. 4
17814/3315711
Printed in U.S.A.

Investigation of the Inhibitory Effects of Various Drugs on the Hepatic Uptake of Fexofenadine in Humans

Soichiro Matsushima, Kazuya Maeda, Naoki Ishiguro, Takashi Igarashi, and Yuichi Sugiyama

Graduate School of Pharmaceutical Sciences, the University of Tokyo, Hongo, Bunkyo-ku, Tokyo, Japan (S.M., K.M., Y.S.); and Department of Pharmacokinetics and Non-Clinical Safety, Kawanishi Pharma Research Institute, Nippon Boehringer Ingelheim Co., Ltd., Yato, Kawanishi, Hyogo, Japan (N.I., T.I.)

Received July 25, 2007; accepted January 4, 2008

ABSTRACT:

Fexofenadine (FEX), an H_1-receptor antagonist, is eliminated from the liver mainly in an unchanged form. Our previous study suggested that organic anion-transporting polypeptide (OATP) 1B3 contributes mainly to the hepatic uptake of FEX. On the other hand, a clinical report demonstrated that a T521C mutation of OATP1B1 increased its plasma area under the curve. Several compounds are reported to have a drug interaction with FEX, and some of this may be caused by the inhibition of its hepatic uptake. We determined which transporters are involved in the hepatobiliary transport of FEX by using double transfectants and examined whether clinically reported drug interactions with FEX could be explained by the inhibition of its hepatic uptake. Vectorial basal-to-apical transport of FEX was observed in double transfectants expressing OATP1B1/multidrug resistance-associated protein 2 (MRP2) and OATP1B3/MRP2, suggesting that OATP1B1 as well as OATP1B3 is involved in the hepatic uptake of FEX and that MRP2 can recognize FEX as a substrate. The inhibitory effects of compounds on FEX uptake in OATP1B3-expressing HEK293 cells were investigated, and the maximal degree of increase in plasma AUC of FEX by drug interaction in clinical situations was estimated. As a result, cyclosporin A and rifampicin were found to have the potential to interact with OATP1B3-mediated uptake at clinical concentrations. From these results, most of the reported drug interaction cannot be explained by the inhibition of hepatic uptake of FEX, and different mechanisms such as the inhibition of intestinal efflux should be considered.

AQ: A

Fexofenadine (FEX) is an orally active nonsedating histamine H_1-receptor antagonist for the treatment of allergic rhinitis and chronic idiopathic urticaria. After oral administration of [^{14}C]FEX to healthy volunteers, 80% of the total dose was recovered in feces and 12% in urine in an unchanged form (Lippert et al., 1995). Because the absolute oral bioavailability of FEX was reported to be 33% (product information; Hoechst Marion, Roussel, Laval, QC, Canada), about two-thirds of bioavailable FEX is estimated to be excreted into bile. Accordingly, some drug transporters in the liver are major determinants for the clearance of FEX from systemic blood.

Fabb

The first step in the process of elimination from the liver is hepatic uptake across the sinusoidal membrane. Accumulated evidence has supported the idea that organic anion-transporting polypeptide (OATP) 1B1 (OATP1B1) and OATP1B3 play major roles in the hepatic uptake of a wide variety of compounds including endogenous compounds and clinically important drugs such as HMG-CoA reductase inhibitors (statins) (Hagenbuch and Meier, 2004). Shimizu et al. (2005) have demonstrated that OATP1B3 contributes mainly to the hepatic uptake of FEX using transporter-expressing HEK293 cells. On the other hand, a recent clinical report has shown that the genetic polymorphism of OATP1B1 (T521C), which was reported to decrease the transport clearance, increased the plasma AUC of FEX (Niemi et al., 2005). These results suggested that OATP1B1 as well as OATP1B3 is involved in the uptake of FEX into human liver.

Many clinical reports have indicated the interaction between FEX and several drugs. Among them, itraconazole (Shon et al., 2005; Shimizu et al., 2006a,b; Uno et al., 2006), ketoconazole (Common Technical Document for the Registration of Pharmaceuticals for Human Use); azithromycin (Gupta et al., 2001), erythromycin (Common Technical Document), ritonavir, lopinavir (van Heeswijk et al., 2006), verapamil, and probenecid (Yasui-Furukori et al., 2005) increased the plasma AUC of FEX, whereas rifampicin (Hamman et al., 2001) decreased it. One of the possible mechanisms for the increase of its AUC is the inhibition of multidrug resistance 1 (MDR1/ABCB1) in the small intestine by the concomitantly administered compounds. Vectorial transport was reported to be observed in MDR1-expressing LLC-PK1 cells but not in parent cells (Cvetkovic et al., 1999). Furthermore, after oral administration of FEX, the plasma AUC in Mdr1a/1b$^{-/-}$ mice was approximately 6 times greater than that in FVB mice, whereas after i.v. administration of FEX, there was no

AQ: B

This study was supported by Industrial Technology Research Grant Program in 2006 from New Energy and Industrial Technology Development Organization of Japan and by Health and Labor Sciences Research Grants from the Ministry of Health, Labor, and Welfare for the Research on Regulatory Science of Pharmaceuticals and Medical Devices.

Article, publication date, and citation information can be found at http://dmd.aspetjournals.org.

doi:10.1124/dmd.107.017814.

ABBREVIATIONS: FEX, fexofenadine; OATP organic anion-transporting polypeptide; AUC, area under the curve; MDR/Mdr, multidrug resistance; MRP, multidrug resistance-associated protein; EG, estradiol-17β-D-glucuronide; MDCK, Madin-Darby canine kidney; LC/MS, liquid chromatography/mass spectrometry.

difference in the pharmacokinetics of FEX between FVB and Mdr1a/1b$^{-/-}$ mice, suggesting that Mdr1a/1b in the small intestine limits the absorption of FEX (Tahara et al., 2005). Because most of the compounds that clinically increase the AUC of FEX have the ability to inhibit MDR1 function, these are likely to increase the intestinal absorption of FEX by the inhibition of MDR1 in the small intestine.

However, considering that the main elimination pathway of FEX is biliary excretion of the unchanged form (Lippert et al., 1995), it is also possible that drug interaction with FEX is caused by the inhibition of its hepatic uptake. Hirano et al. (2006) performed detailed investigations to see whether the inhibitory effects of various compounds on OATP1B1-mediated uptake of pitavastatin were clinically relevant. However, the inhibition potencies of several compounds for OATP1B3-mediated uptake have not yet been clarified.

Therefore, the purpose of this study was to determine which transporters are involved in the hepatobiliary transport of FEX and explore which instances can be explained by the inhibition of its hepatic uptake among clinically reported drug interactions. We identified the transporters that can transport FEX by using double transfectants expressing OATP1B1/multidrug resistance-associated protein (MRP) 2 and OATP1B3/MRP2 (Matsushima et al., 2005). It has often been found that the transcellular transport assay using double transfectants is more sensitive in detecting transporter-mediated transport than the uptake assay in single transporter-expressing cells. Thus, we analyzed the inhibitory effects of several compounds that are reported to interact with FEX in clinical situations on FEX uptake in OATP1B3-expressing HEK293 cells and compared the in vitro inhibition constant (K_i) for OATP1B3 with that for OATP1B1 obtained from a previous study (Hirano et al., 2006). After that, to determine whether the inhibition of FEX uptake by several compounds is clinically relevant, we also estimated the maximal degree of increase in the plasma AUC of FEX by considering the maximal unbound concentration of inhibitors at the inlet to the liver estimated by an established method for this calculation (Ito et al., 1998).

Materials and Methods

Materials. [^{3}H]Estradiol-17β-D-glucuronide (EG) (1.6 TBq/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). FEX hydrochloride was purchased from Toronto Research Chemicals (North York, ON, Canada). All other chemicals and reagents were of analytical grade and commercially available.

Cell Culture. MDCKII cells expressing OATP1B1/MRP2, OATP1B3/MRP2, OATP1B1, OATP1B3, and MRP2 and vector-transfected control MDCKII cells have been constructed previously (Matsushima et al., 2005, Ishiguro et al., submitted for publication). OATP1B3-expressing HEK293 cells and vector-transfected control cells were also constructed previously (Hirano et al., 2004). Transporter-expressing or vector-transfected MDCKII and HEK293 cells were grown in Dulbecco's modified Eagle's medium (low glucose) (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO) and 1% antibiotic-antimycotic solution (Sigma-Aldrich) at 37°C under 5% CO_2 and 95% humidity.

Transcellular Transport Study. The transcellular transport study was performed as reported previously (Matsushima et al., 2005). Briefly, MDCKII cells were grown on Transwell membrane inserts (6.5 mm diameter, 0.4 μm pore size; Corning Coster, Bodenheim, Germany) at confluence for 7 days, and the medium was replaced with Dulbecco's modified Eagle's medium supplemented with 5 mM sodium butyrate 2 days before the transport study to induce the expression of exogenous transporter. In the transport assay, cells were first washed with Krebs-Henseleit buffer (118 mM NaCl, 23.8 mM $NaHCO_3$, 4.83 mM KCl, 0.96 mM KH_2PO_4, 1.20 mM $MgSO_4$, 12.5 mM HEPES, 5.0 mM glucose, and 1.53 mM $CaCl_2$ adjusted to pH 7.4) at 37°C and preincubated with Krebs-Henseleit buffer for 10 min. Subsequently, substrates were added in Krebs-Henseleit buffer either to the apical compartment (250 μl) or to the basolateral compartment (1 ml). After a designated period, 50 or 100 μl of medium was taken from the opposite side to the added substrate. Using FEX as a substrate, 50-μl aliquots were used for LC/MS quantification as described below. At the end of the experiments, cells were washed with ice-cold Krebs-Henseleit buffer and solubilized in 500 μl of 0.2 N NaOH. After addition of 100 μl of 1 N HCl, 50-μl aliquots were used to determine protein concentrations by the method of Lowry et al. (1951) with bovine serum albumin as a standard.

Uptake Study Using OATP1B3-Expressing HEK293 Cells. Cells were seeded in 12-well plates coated with poly-L-lysine/poly-L-ornithine (Sigma-Aldrich) at a density of 1.5×10^5 cells/well. After 2 days, the cell culture medium was replaced with culture medium supplemented with 5 mM sodium butyrate 24 h before the transport assay to induce the expression of exogenous transporters. The transport study was carried out as described previously (Sugiyama et al., 2001). Uptake was initiated by adding Krebs-Henseleit buffer containing FEX. All of the procedures were performed at 37°C. The uptake was terminated at a designated time by adding ice-cold Krebs-Henseleit buffer after removal of the incubation buffer. Then, cells were washed twice with 1 ml of ice-cold buffer, solubilized in 500 μl of 0.2 N NaOH, and kept overnight at room temperature. Using EG as a substrate, aliquots (300 μl) were transferred to vials after addition of 100 μl of 1 N HCl. Using FEX as a substrate, aliquots (240 μl) were used for LC/MS quantification as described below. The remaining 50 μl of the aliquots of cell lysate were used to determine the protein concentration by the method of Lowry et al. (1951) with bovine serum albumin as a standard.

Quantification of FEX by LC/MS. The aliquots (50 μl) obtained from the transcellular transport study were precipitated with 200 μl of methanol containing 10 nM midazolam as an internal standard, whereas the aliquots (240 μl) obtained from the uptake study were precipitated with 480 μl of methanol containing 50 nM midazolam as an internal standard. After centrifugation (15,000g for 10 min at 4°C) of the mixture, 50 μl of 0.05% formic acid was added to 50 μl of supernatant. The samples obtained were subjected to LC/MS analysis to determine the concentration of FEX. An LC/MS-2010 EV equipped with a Prominence LC system (Shimadzu, Kyoto, Japan) was used for the analysis. The samples were separated on a CAPCELL PAK C18 MG column (3 μm, 4.6 mm i.d., 75 mm; Shiseido, Tokyo, Japan) in binary gradient mode. For the mobile phase, 0.05% formic acid and methanol were used. The methanol concentration was initially 48%, which was then linearly increased up to 61.5% over 4.5 min. Finally, the column was reequilibrated in a methanol concentration of 48% for 3 min. The total run time was 7.5 min. By using this method, FEX was eluted at 4.1 min and midazolam at 2.8 min. In the mass analysis, FEX and midazolam were detected at mass-to-charge ratios of 502.3 and 326.1 under positive ionization conditions. The interface voltage was 3.5 kV, and the nebulizer gas (N_2) flow was 1.5 liters/min. The heat block and curved desolvation line temperatures were 200 and 150°C, respectively.

Kinetic Analyses. Ligand uptake in transporter-expressing cells was expressed as the uptake volume (microliters per milligram of protein), given as the amount associated with the cells divided by its initial concentration in the incubation medium. Transporter-specific uptake was obtained by subtracting the uptake into vector-transfected cells from that into transporter-expressing cells. Inhibition constants (K_i) of a series of compounds could be calculated by the following equation if the substrate concentration was low enough compared with its K_m value:

$$CL_{+I} = \frac{CL}{1 + I/K_i} \quad (1)$$

where CL represents the uptake clearance in the absence of inhibitor, CL_{+I} represents the uptake clearance in the presence of inhibitor, and I represents the inhibitor concentration. When the data were fitted to determine the K_i value, the input data were weighted as the reciprocal of the observed values. The Damping Gauss-Newton method algorithm was used with a MULTI software program (Yamaoka et al., 1981) to fit the data.

Prediction of Clinical Drug-Drug Interactions between FEX and Various Compounds. The degree of inhibition of uptake via OATP1B1 and OATP1B3 in humans was estimated by calculating the following R value, which represents the ratio of the uptake clearance in the absence of inhibitor to that in its presence.

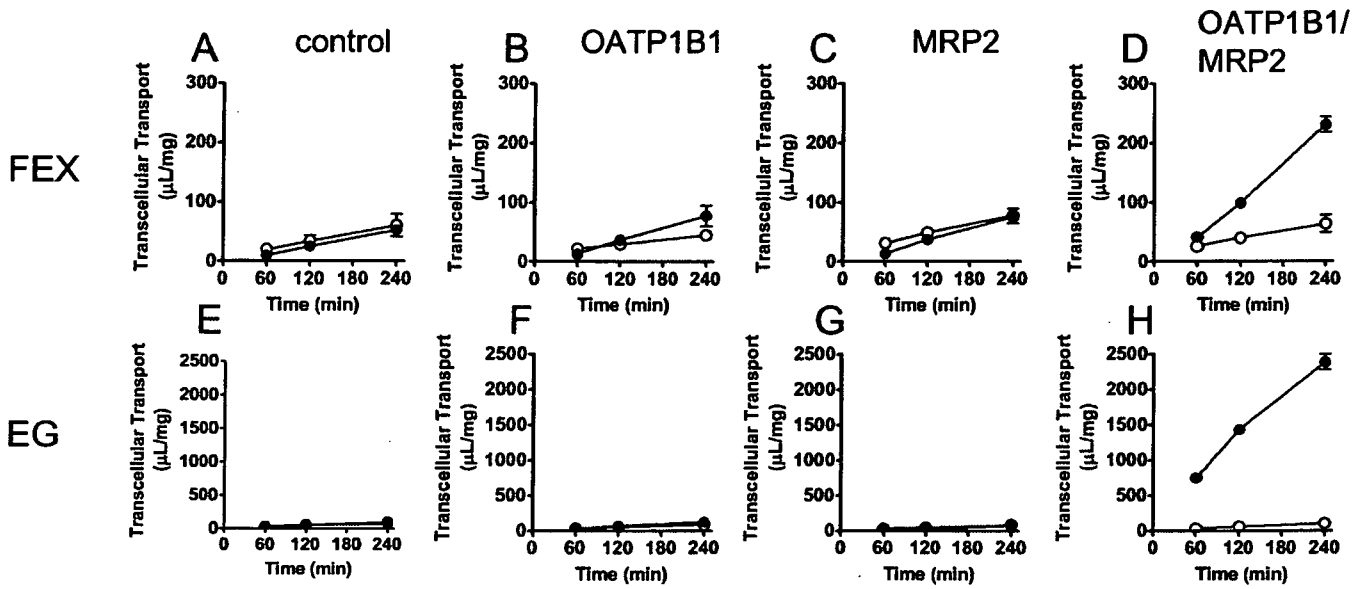

FIG. 1. Time profiles for the transcellular transport of FEX and EG across MDCKII cell monolayers expressing OATP1B1 and/or MRP2. The transcellular transport of 5 μM FEX (A–D) and 0.1 μM EG (E–H) across MDCKII cell monolayers expressing OATP1B1 (B and F), MRP2 (C and G), and both OATP1B1 and MRP2 (D and H) was compared with that across the vector-transfected control MDCKII cell monolayer (A and E). Otranscellular transport in the apical-to-basal direction; Otranscellular transport in the basal-to-apical directions. These data were obtained from three independent experiments, and each experiment was performed in triplicate. Each point with vertical bar represents the mean and S.D. Where a vertical bar is not shown, the S.D. was contained within the limits of the symbol.

$$R = 1 + \frac{f_u \cdot I_{in,max}}{K_i} \quad (2)$$

where f_u represents the protein unbound fraction of the inhibitor in blood and $I_{in,max}$ represents the estimated maximal blood concentration of the inhibitor at the inlet to the liver. The K_i value for OATP1B3 was obtained from the present in vitro study using OATP1B3-expressing HEK293 cells described above, and the K_i value for OATP1B1 is quoted from the previous reports in which pitavastatin was used as a substrate of OATP1B1 (Hirano et al., 2006). For estimation of the R value, $I_{in,max}$ was calculated by the method of Ito et al. (1998) as shown by

$$I_{in,max} = I_{max} + \frac{F_a \cdot dose \cdot k_a}{Q_h} \quad (3)$$

where I_{max} represents the reported value for the maximal blood concentration of the inhibitor in the systemic circulation in clinical situations, F_a represents the fraction absorbed from the intestine of the inhibitor, k_a is the absorption rate constant in the intestine, and Q_h represents the hepatic blood flow rate in humans (1610 ml/min) (Ito et al., 1998). To estimate the maximal $I_{in,max}$, F_a was set at 1, k_a was set at 0.1 ml^{-1} [minimum gastric emptying time (10 min)], and the blood-to-plasma concentration ratio was assumed to be 1.

Results

Transcellular Transport of FEX and EG across the MDCKII Cell Monolayer. The transcellular transport of 5 μM FEX and 0.1 μM EG across the MDCKII monolayer was determined. The basal-to-apical transport of FEX was approximately 3.6 times higher than that in the opposite direction in OATP1B1/MRP2 double transfectants (Fig. 1D), whereas no difference in basal-to-apical transcellular transport of FEX could be observed in vector-transfected control cells and single transfectants expressing OATP1B1 or MRP2 (Fig. 1, A–C). On the other hand, the basal-to-apical transport of EG was approximately 22 times higher than that in the opposite direction in OATP1B1/MRP2 double transfectants (Fig. 1H), whereas we could not see any basal-to-apical transcellular transport of EG in vector-transfected control cells and single transfectants expressing OATP1B1 or MRP2 (Fig. 1, E–G). The basal-to-apical transport of FEX was approximately 2.9 times higher than that in the opposite direction in OATP1B3/MRP2 double transfectants (Fig. 2D), whereas the difference in each directional transport of EG was less than 2 times that in vector-transfected control cells and single transfectants expressing OATP1B3 or MRP2 (Fig. 2, A–C). As a positive control, the basal-to-apical transport of EG was approximately 3.0 times higher than that in the opposite direction in OATP1B3/MRP2 double transfectants (Fig. 2H), whereas no significant difference in basal-to-apical transcellular transport of EG was less than 2 times that in vector-transfected control cells and single transfectants expressing OATP1B3 or MRP2 (Fig. 2, E–G).

Inhibitory Effects of Various Drugs on OATP1B3-Mediated Uptake of FEX. The inhibitory effects of various drugs on the uptake of FEX were examined using OATP1B3-expressing HEK293 cells (Fig. 3). Some of the drugs we tested were reported to cause drug-drug interactions with FEX in clinical situations. Most of the compounds could inhibit OATP1B3-mediated FEX uptake. On the other hand, even 100 μM fluconazole, 30 μM itraconazole, and 100 μM cimetidine did not significantly inhibit FEX uptake (Fig. 3, C, D, and N). We also obtained the protein unbound fraction in blood (f_u) from the literature and calculated the estimated maximal concentration at the inlet to the liver ($I_{in,max}$) of the inhibitors. K_i values of various compounds for OATP1B3 obtained in the present study and the ratio of the uptake clearance in the absence of inhibitor to that in its presence (R value) are summarized in Table 1. Among several drugs we tested, only the R values of cyclosporin A and rifampicin for OATP1B3-mediated uptake exceeded 2.0. The K_i values of various compounds for OATP1B1 obtained in the previous study and the respective R values are also shown in Table 1 (Hirano et al., 2006). The K_i values of several compounds in the uptake mediated by OATP1B1 and OATP1B3 were not so different. However, the K_i values of clarithromycin and ritonavir for OATP1B1-mediated uptake of pitavastatin were more than 5-fold less compared with that for OATP1B3-mediated uptake of FEX.

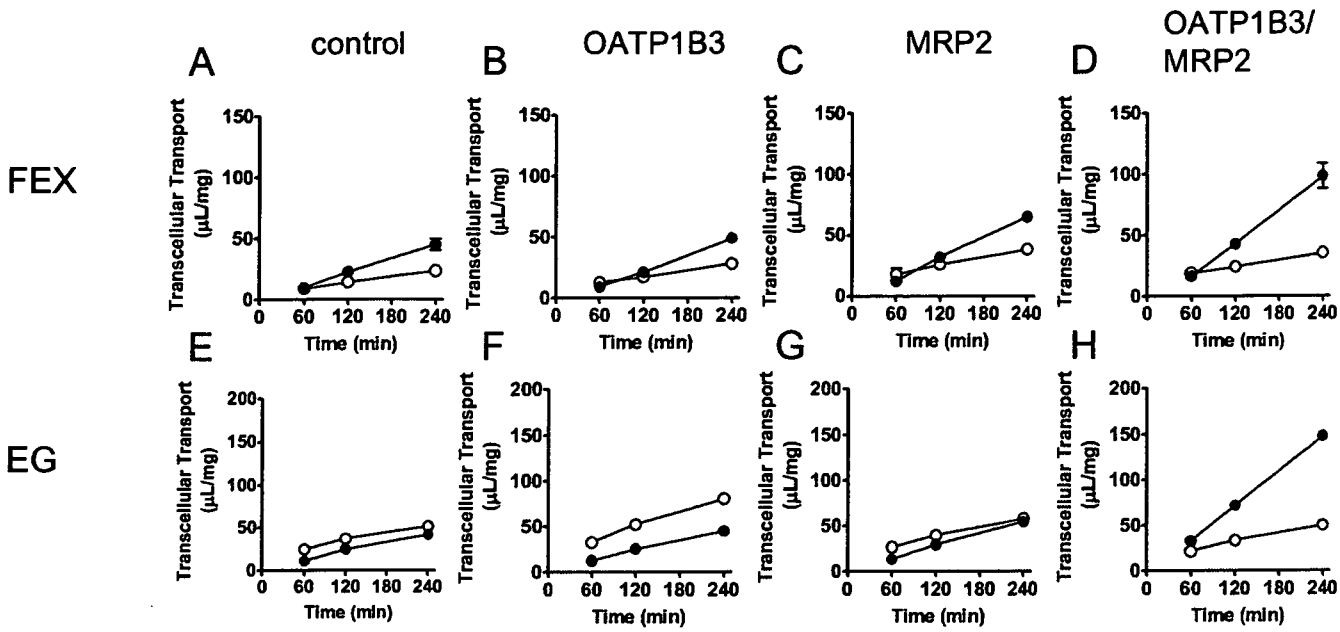

FIG. 2. Time profiles for the transcellular transport of FEX and EG across MDCKII cell monolayers expressing OATP1B3 and/or MRP2. The transcellular transport of 5 μM FEX (A–D) and 0.1 μM EG (E–H) across MDCKII cell monolayers expressing OATP1B3 (B and F), MRP2 (C and G), and both OATP1B3 and MRP2 (D and H) was compared with that across the control MDCKII cell monolayer (A and E). Otranscellular transport in the apical-to-basal direction; Otranscellular transport in the basal-to-apical directions, respectively. These data were obtained from three independent experiments, and each experiment was performed in triplicate. Each point with vertical bar represents the mean and S.D. Where a vertical bar is not shown, the S.D. was contained within the limits of the symbol.

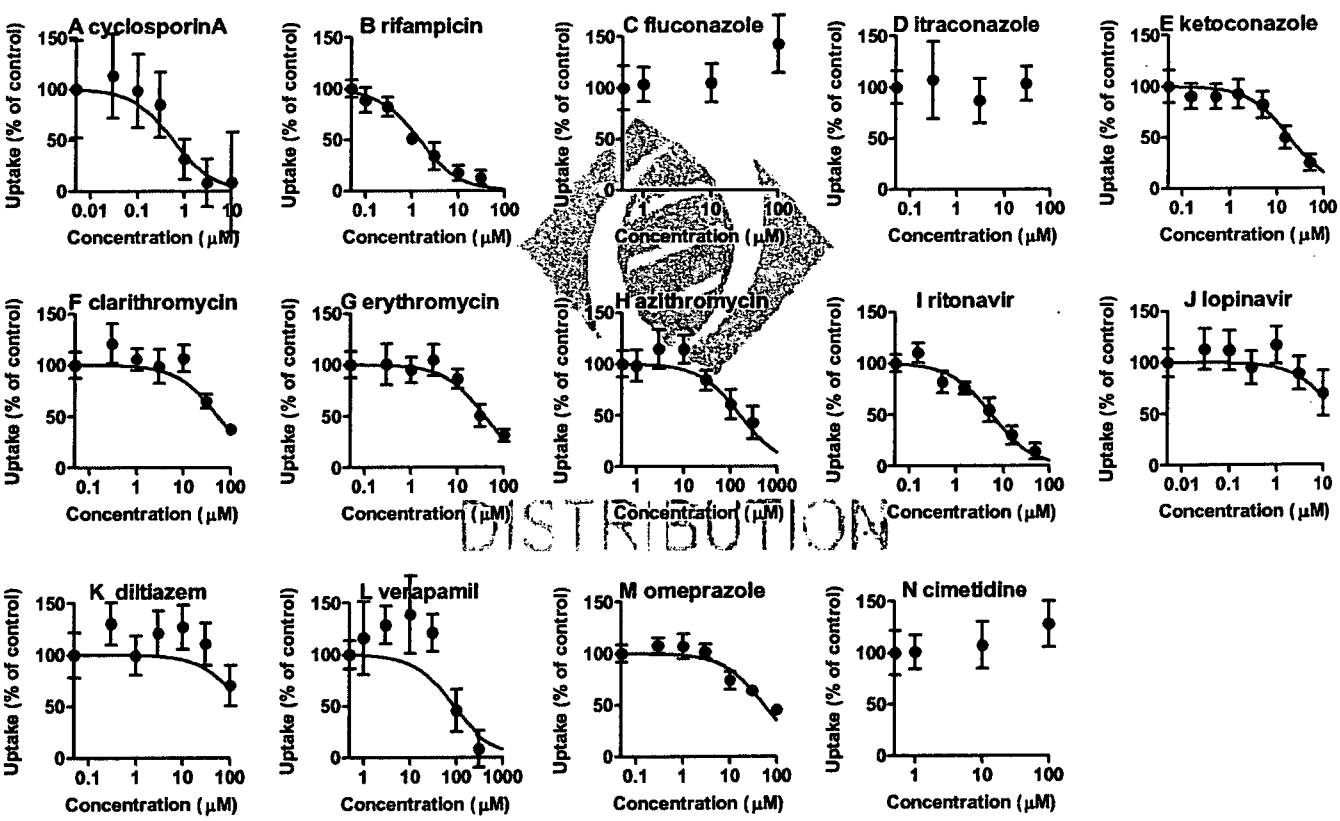

FIG. 3. Inhibitory effects of various drugs on the OATP1B3-mediated uptake of FEX. The OATP1B3-mediated uptake of FEX (10 μM) was determined in the absence or presence of inhibitors cyclosporin A (A), rifampicin (B), fluconazole (C), itraconazole (D), ketoconazole (E), clarithromycin (F), erythromycin (G), azithromycin (H), ritonavir (I), lopinavir (J), diltiazem (K), verapamil (L), omeprazole (M), and cimetidine (N) using OATP1B3-expressing HEK293 cells. These data were obtained from three independent experiments, and each experiment was performed in triplicate. Each point with vertical bar represents the mean and S.D. Where a vertical bar is not shown, the S.D. was contained within the limits of the symbol. Each solid line represents the fitting curve obtained by nonlinear regression analysis.

TABLE 1

Comparison of K_i values of inhibitors for OATP1B1-mediated uptake of pitavastatin and OATP1B3-mediated uptake of FEX

K_i values are expressed as the mean ± computer-calculated S.D. The inhibitors, which are shown in boldface, increased the plasma AUC in the previous clinical studies. Data for dosages, I_{max}, and f_u are from product information of each drug except for the data with footnotes. R and $I_{in,max}$ values are calculated according to eq. 2 and eq. 3, respectively (see *Materials and Methods*).

Inhibitor	Dosage	I_{max}	$I_{in,max}$	f_u	OATP1B3		OATP1B1	
					K_i	R	K_i[a]	R
	mg	μM	μM		μM		μM	
Cyclosporin A	100	0.596	5.76	0.10[b]	0.573 ± 0.172	2.01	0.242 ± 0.029	3.55
Rifampicin	600[c]	7.90[c]	53.2	0.11[c]	1.45 ± 0.28	5.03	0.477 ± 0.030	13.3
Fluconazole	100	8.62	28.9	0.89[c]	>100	<1.26	>100	<1.26
Itraconazole	100	0.0792	8.88	0.002[c]	>30	1.00	>100	1.00
Ketoconazole	200[c]	3.20[c]	26.6	0.01[c]	18.5 ± 3.0	1.01	19.2 ± 3.9	1.01
Clarithromycin	400	1.86	35.1	0.54[c]	53.6 ± 15.9	1.42	8.26 ± 0.54	3.29
Erythromycin	200	1.12	18.0	0.16[c]	38.3 ± 7.7	1.08	11.4 ± 2.1	1.25
Azithromycin	1200	0.881	100	0.84	161 ± 7.7	1.52	N.D.	
Ritonavir	800	28.5	97.4	0.02[c]	5.64 ± 1.39	1.35	0.781 ± 0.048	3.49
Lopinavir	400	15.2	54.8	0.015	18.4 ± 7.2	1.04	N.D.	
Probenecid	2000[d]	52.0[d]	487	0.10[e]	130 ± 40[e]	1.37	76.2 ± 7.1	1.64
Diltiazem	100	0.0536	15.0	0.22[c]	193 ± 112	1.03	>100	<1.03
Verapamil	80	0.190	11.1	0.10[c]	89.5 ± 52.9	1.01	51.6 ± 15.9	1.02
Omeprazole	20	1.18	4.77	0.03	53.9 ± 14.3	1.00	N.D.	
Cimetidine	200	2.75	52.0	0.81[c]	>100	<1.42	>300	<1.14

N.D., not determined.
[a] K_i values of inhibitors for the OATP1B1-mediated uptake of pitavastatin were determined previously (Hirano et al., 2006).
[b] Data for protein unbound fraction of cyclosporin A are from a previous report (Lemaire and Tillement, 1982).
[c] These data are from Hardman and Limbard (2001).
[d] Data for dosages and I_{max} of probenecid are from a previous report (Selen et al., 1982).
[e] K_i values of probenecid were determined previously (Tahara et al., 2006).

Discussion

To determine which transporters are involved in the hepatobiliary transport of FEX, we investigated the transcellular transport of FEX using OATP1B1/MRP2 and OATP1B3/MRP2 double-transfected cells. Furthermore, to investigate whether the inhibition of FEX hepatic uptake by several drugs is clinically relevant, the inhibition constants of several drugs for OATP1B3-mediated FEX uptake obtained from in vitro analyses were determined, and the maximal degrees of increase in the plasma AUC through drug interactions were calculated using estimated maximal protein unbound concentrations of inhibitors at the inlet to the liver.

In the transcellular transport study using double-transfected cells, we observed the basal-to-apical vectorial transport of FEX not only in OATP1B3/MRP2 but also in OATP1B1/MRP2double-transfected cells (Figs. 1 and 2). Our previous report indicated that OATP1B3 contributes mainly to the hepatic uptake of FEX in humans and that OATP1B1-mediated uptake was not statistically significant although the uptake in OATP1B1-expressing cells was slightly larger than that in control cells (Shimizu et al., 2005). The involvement of OATP1B1 in FEX uptake in humans was also supported by the recent clinical report demonstrating that the genetic polymorphism of OATP1B1 (T521C) increased the plasma concentration of FEX (Niemi et al., 2005). The apparently conflicting results obtained from the present transcellular transport study and the previous uptake study may be caused by the difference in the sensitivity for the detection of the transport. We found that a transcellular transport assay using a double transfectant is more sensitive in detecting the transporter-mediated transport than an uptake assay in single transporter-expressing cells (Sasaki et al., 2002; Matsushima et al., 2005). For example, the ratio of the basal-to-apical transport of pravastatin to that in the opposite direction was 3.3, whereas it barely estimates the kinetics of pravastatin transport in OATP1B1-expressing HEK293 cells because of its small OATP1B1-mediated uptake (Matsushima et al., 2005). Therefore, these results suggest a significant contribution of OATP1B1 as well as OATP1B3 to the hepatic uptake of FEX. Further evaluation is required for the determination of the precise relative contribution of OATP1B1 and OATP1B3 to the hepatic uptake of FEX in humans.

The finding presented here is the first demonstration that human MRP2 can recognize FEX as a substrate. MRP2 is expressed in the apical membrane of the liver, kidney, and intestine. An in vivo infusion study using Eisai hyperbilirubinemic rats, which are Mrp2-deficient, revealed that Mrp2 is not important for the biliary excretion of FEX (Tahara et al., 2005). However, it is still possible that MRP2 will play an important role in the disposition of FEX in humans. This situation is very similar to that reported for pitavastatin (Hirano et al., 2005). The transcellular transport study using OATP1B1/MRP2 double-transfected cells indicated that pitavastatin could be transported by MRP2. However, the biliary excretion of pitavastatin in Eisai hyperbilirubinemic rats was not changed compared with that in control rats. Moreover, there are some reports that show species differences in the expression and function of MRP2 (Ishizuka et al., 1999; Ninomiya et al., 2005, 2006; Takekuma et al., 2007). These may also indicate the species difference in the relative contribution of efflux transporters to the biliary excretion of compounds. The methodology to determine the contribution of efflux transporters in human liver needs to be established by checking the effect of transporter-specific inhibitors on the efflux of compounds in membrane vesicles prepared from human liver or sandwich-cultured human hepatocytes.

Several reports regarding drug-drug interactions with FEX have been published. It is generally believed that one of the major mechanisms of the reported drug-drug interactions between FEX and concomitantly administered drugs is the inhibition of MDR1-mediated efflux in the small intestine, which plays an important role in limiting the entry of FEX into circulating blood (Tahara et al., 2005). However, some of the reported cases are thought to be caused by mechanisms other than the inhibition of MDR1. A regional perfusion study showed that ketoconazole and verapamil did not have a significant effect on the in vivo intestinal absorption of FEX when coadministered or given as a pretreatment despite increasing the plasma AUC of FEX (Tannergren et al., 2003). Accordingly, the involvement

of other mechanisms in addition to the inhibition of MDR1 has been supposed. One of the candidate mechanisms is considered to reduce the hepatic uptake clearance because the major route of FEX elimination is biliary excretion of the unchanged form. Because FEX is barely metabolized, the apparent intrinsic hepatic clearance is described as follows:

$$CL_{int,\,h} = CL_{uptake} \cdot \frac{CL_{excretion}}{CL_{efflux} + CL_{excretion}} \quad (4)$$

where $CL_{int,\,h}$ represents the apparent intrinsic hepatic clearance, CL_{uptake} represents the hepatic uptake clearance, $CL_{excretion}$ represents the biliary excretion clearance, and CL_{efflux} represents the backflux clearance from liver to blood. According to eq. 4, the change in the hepatic uptake clearance always directly affects the overall intrinsic hepatic clearance. The present study indicates that both OATP1B1 and OATP1B3 contribute to the hepatic uptake of FEX. Previously we reported the inhibitory effects of various drugs on OATP1B1-mediated uptake and their clinical relevance to drug-drug interaction (Hirano et al., 2006). However, this kind of systematic investigation for OATP1B3 has not been conducted. Therefore, the inhibitory effects of various drugs on OATP1B3-mediated uptake were determined. Among several compounds we tested, the R values of cyclosporin A and rifampicin for OATP1B3 as well as for OATP1B1 exceeded 2.0 (Table 1). To date, we have not been able to find a published report regarding a drug-drug interaction between FEX and cyclosporin A. Many clinical reports have indicated that cyclosporin A increases the AUC of a variety of substrates of OATP transporters, particularly HMG-CoA reductase inhibitors (Shitara et al., 2005). Although cyclosporin A is known as a clinically relevant potent OATP1B1 inhibitor (Shitara et al., 2003), we showed that cyclosporin A can also potently inhibit OATP1B3-mediated uptake. Accordingly, it is necessary to pay attention to not only the OATP1B1- but also the OATP1B3-mediated drug-drug interaction between FEX and cyclosporin A in clinical situations. Repetitive administration of rifampicin reduced the plasma AUC of FEX in a previous clinical study (Hamman et al., 2001). This report apparently conflicts with the present results in which rifampicin inhibited the OATP1B3-mediated uptake. However, rifampicin is a well known pregnane X receptor-mediated inducer and increases the expression level of MDR1 in the small intestine (Schuetz et al., 1996). Therefore, in this case, repeated dosing of rifampicin increased the expression level of MDR1 in the small intestine, which masked its inhibitory effects on the OATP1B3-mediated uptake of FEX. This concept is supported by the recent report from Lam et al. (2006) indicating that drugs should be administered 1 day after the final dose of rifampicin to minimize potential inhibitory effects of OATP transporters in the induction study (Lam et al., 2006).

When we compared K_i values for OATP1B1 with those for OATP1B3 K_i values for OATP1B1 and OATP1B3 were within the range of a 5-fold difference, except for clarithromycin and ritonavir, suggesting that the inhibitory potency of compounds for OATP1B1-mediated transport can be considered similar to that for OATP1B3-mediated transport. A specific inhibitor for each individual transporter is very useful for determining the contribution of each transporter to the overall membrane transport. Although EG and estrone-3-sulfate are recognized as selective inhibitors for OATP1B1/OATP1B3 and OATP1B1/OATP2B1, respectively (Hirano et al., 2006), unfortunately, specific inhibitors for OATP1B3 have not yet been identified. Because of the high homology and overlapping substrate specificities between OATP1B1 and OATP1B3, the use of in silico screening with a ligand-based drug design approach may be necessary to search for the selective inhibitors for OATP1B3 (Hirano et al., 2004).

Hirano et al. (2006) have indicated that cyclosporin A, rifampicin, clarithromycin, and ritonavir (R value for OATP1B1 >2.0) have a potential to interact with OATP1B1-mediated transport of pitavastatin in clinical situations (Hirano et al., 2006). Although the K_i values for OATP1B1-mediated uptake were determined by using pitavastatin as a substrate because of no significant uptake of FEX into OATP1B1-expressing HEK293 cells, if we consider the possible contribution of OATP1B1 and OATP1B3 to the hepatic uptake of FEX, these drugs may also affect the hepatic clearance of FEX. To avoid false-negative predictions of drug-drug interactions, the maximal plasma unbound concentration of inhibitors at the inlet to the liver was calculated using eq. 3, which can overestimate these concentrations (Ito et al., 1998). Therefore, in most cases, a drug-drug interaction caused by inhibition of hepatic uptake of FEX might not occur in clinical situations.

In conclusion, both OATP1B1 and OATP1B3 are involved in the hepatic uptake of FEX, and MRP2 can recognize FEX as a substrate. Among the compounds we tested, cyclosporin A and rifampicin have the potential to inhibit the OATP1B1- and OATP1B3-mediated hepatic uptake of FEX at clinically relevant concentrations. However, most of the reported clinical drug-drug interactions cannot be explained simply by the inhibition of hepatic uptake of FEX, and other mechanisms should be taken into account (e.g., inhibition of MDR1-mediated efflux in small intestine).

Acknowledgments. We thank Dr. Junko Iida and Futoshi Kurotobi (Shimadzu Corporation, Kyoto, Japan) for technical support involving the LC/MS system. We also thank Atsushi Ose for providing valuable comments on the LC/MS system.

References

Cvetkovic M, Leake B, Fromm MF, Wilkinson GR, and Kim RB (1999) OATP and P-glycoprotein transporters mediate the cellular uptake and excretion of fexofenadine. *Drug Metab Dispos* **27**:866–871.

Gupta S, Banfield C, Kantesaria B, Marino M, Clement R, Affrime M, and Batra V (2001) Pharmacokinetic and safety profile of desloratadine and fexofenadine when coadministered with azithromycin: a randomized, placebo-controlled, parallel-group study. *Clin Ther* **23**:451–466.

Hagenbuch B, and Meier PJ (2004) Organic anion transporting polypeptides of the OATP/SLC21 family: phylogenetic classification as OATP/SLCO superfamily, new nomenclature and molecular/functional properties. *Pflugers Arch* **447**:653–665.

Hamman MA, Bruce MA, Haehner-Daniels BD, and Hall SD (2001) The effect of rifampin administration on the disposition of fexofenadine. *Clin Pharmacol Ther* **69**:114–121.

Hardman JG and Limbard LE, editors (2001) *The Pharmacological Basis of Therapeutics*, 10th ed. McGraw-Hill, New York.

Hirano M, Maeda K, Matsushima S, Nozaki Y, Kusuhara H, and Sugiyama Y (2005) Involvement of BCRP (ABCG2) in the biliary excretion of pitavastatin. *Mol Pharmacol* **68**:800–807.

Hirano M, Maeda K, Shitara Y, and Sugiyama Y (2004) Contribution of OATP2 (OATP1B1) and OATP8 (OATP1B3) to the hepatic uptake of pitavastatin in humans. *J Pharmacol Exp Ther* **311**:139–146.

Hirano M, Maeda K, Shitara Y, and Sugiyama Y (2006) Drug-drug interaction between pitavastatin and various drugs via OATP1B1. *Drug Metab Dispos* **34**:1229–1236.

Ishizuka H, Konno K, Shiina T, Naganuma H, Nishimura K, Ito K, Suzuki H, and Sugiyama Y (1999) Species differences in the transport activity for organic anions across the bile canalicular membrane. *J Pharmacol Exp Ther* **290**:1324–1330.

Ito K, Iwatsubo T, Kanamitsu S, Ueda K, Suzuki H, and Sugiyama Y (1998) Prediction of pharmacokinetic alterations caused by drug-drug interactions: metabolic interaction in the liver. *Pharmacol Rev* **50**:387–412.

Lam JL, Smigaris SB, Okochi H, and Benet LZ (2006) Elucidating the effect of final-day dosing of rifampin in induction studies on hepatic drug disposition and metabolism. *J Pharmacol Exp Ther* **319**:861–870.

Lippert C, Lang J, Brown P, and Burmaster S (1995) Mass balance and pharmacokinetics of MDL 16,455A in the healthy, male volunteers. *Pharm Res* **12**:S-390.

Lowry OH, Rosebrough NJ, Farr AL and Randall RJ (1951) Protein measurement with Folin phenol reagent. *J Biol Chem* **193**:265–267.

Matsushima S, Maeda K, Kondo C, Hirano M, Sasaki M, Suzuki H, and Sugiyama Y (2005) Identification of the hepatic efflux transporters of organic anions using double-transfected Madin-Darby canine kidney II cells expressing human organic anion-transporting polypeptide 1B1 (OATP1B1)/multidrug resistance-associated protein 2, OATP1B1/multidrug resistance 1, and OATP1B1/breast cancer resistance protein. *J Pharmacol Exp Ther* **314**:1059–1067.

Niemi M, Kivisto KT, Hofmann U, Schwab M, Eichelbaum M, and Fromm MF (2005) Fexofenadine pharmacokinetics are associated with a polymorphism of the SLCO1B1 gene (encoding OATP1B1). *Br J Clin Pharmacol* **59**:602–604.

Ninomiya M, Ito K, Hiramatsu R, and Horie T (2006) Functional analysis of mouse and monkey multidrug resistance-associated protein 2 (Mrp2). *Drug Metab Dispos* **34**:2056–2063.

Ninomiya M, Ito K, and Horie T (2005) Functional analysis of dog multidrug resistance-associated protein 2 (Mrp2) in comparison with rat Mrp2. *Drug Metab Dispos* **33**:225–232.

Sasaki M, Suzuki H, Ito K, Abe T, and Sugiyama Y (2002) Transcellular transport of organic anions across a double-transfected Madin-Darby canine kidney II cell monolayer expressing both human organic anion-transporting polypeptide (OATP2/SLC21A6) and multidrug resistance-associated protein 2 (MRP2/ABCC2). *J Biol Chem* **277**:6497–6503.

Schuetz EG, Beck WT, and Schuetz JD (1996) Modulators and substrates of P-glycoprotein and cytochrome P4503A coordinately up-regulate these proteins in human colon carcinoma cells. *Mol Pharmacol* **49**:311–318.

Selen A, Amidon GL, and Welling PG (1982) Pharmacokinetics of probenecid following oral doses to human volunteers. *J Pharm Sci* **71**:1238–1242.

Shimizu M, Fuse K, Okudaira K, Nishigaki R, Maeda K, Kusuhara H, and Sugiyama Y (2005) Contribution of OATP (organic anion-transporting polypeptide) family transporters to the hepatic uptake of fexofenadine in humans. *Drug Metab Dispos* **33**:1477–1481.

Shimizu M, Uno T, Sugawara K, and Tateishi T (2006a) Effects of itraconazole and diltiazem on the pharmacokinetics of fexofenadine, a substrate of P-glycoprotein. *Br J Clin Pharmacol* **61**:538–544.

Shimizu M, Uno T, Sugawara K, and Tateishi T (2006b) Effects of single and multiple doses of itraconazole on the pharmacokinetics of fexofenadine, a substrate of P-glycoprotein. *Br J Clin Pharmacol* **62**:372–376.

Shitara Y, Itoh T, Sato H, Li AP, and Sugiyama Y (2003) Inhibition of transporter-mediated

hepatic uptake as a mechanism for drug-drug interaction between cerivastatin and cyclosporin A. *J Pharmacol Exp Ther* **304**:610–616.

Shitara Y, Sato H, and Sugiyama Y (2005) Evaluation of drug-drug interaction in the hepatobiliary and renal transport of drugs. *Annu Rev Pharmacol Toxicol* **45**:689–723.

Shon JH, Yoon YR, Hong WS, Nguyen PM, Lee SS, Choi YG, Cha IJ, and Shin JG (2005) Effect of itraconazole on the pharmacokinetics and pharmacodynamics of fexofenadine in relation to the MDR1 genetic polymorphism. *Clin Pharmacol Ther* **78**:191–201.

Sugiyama D, Kusuhara H, Shitara Y, Abe T, Meier PJ, Sekine T, Endou H, Suzuki H, and Sugiyama Y (2001) Characterization of the efflux transport of 17β-estradiol-D-17β-glucuronide from the brain across the blood-brain barrier. *J Pharmacol Exp Ther* **298**:316–322.

Tahara H, Kusuhara H, Fuse E, and Sugiyama Y (2005) P-glycoprotein plays a major role in the efflux of fexofenadine in the small intestine and blood-brain barrier, but only a limited role in its biliary excretion. *Drug Metab Dispos* **33**:963–968.

Tahara H, Kusuhara H, Maeda K, Koepsell H, Fuse E, and Sugiyama Y (2006) Inhibition of oat3-mediated renal uptake as a mechanism for drug-drug interaction between fexofenadine and probenecid. *Drug Metab Dispos* **34**:743–747.

Takekuma Y, Kakiuchi H, Yamazaki K, Miyauchi S, Kikukawa T, Kamo N, Ganapathy V, and Sugawara M (2007) Difference between pharmacokinetics of mycophenolic acid (MPA) in rats and that in humans is caused by different affinities of MRP2 to a glucuronized form. *J Pharm Pharm Sci* **10**:71–85.

Tannergren C, Petri N, Knutson L, Hedeland M, Bondesson U, and Lennernas H (2003) Multiple transport mechanisms involved in the intestinal absorption and first-pass extraction of fexofenadine. *Clin Pharmacol Ther* **74**:423–436.

Uno T, Shimizu M, Sugawara K, and Tateishi T (2006) Lack of dose-dependent effects of itraconazole on the pharmacokinetic interaction with fexofenadine. *Drug Metab Dispos* **34**:1875–1879.

van Heeswijk RP, Bourbeau M, Campbell P, Seguin I, Chauhan BM, Foster BC, and Cameron DW (2006) Time-dependent interaction between lopinavir/ritonavir and fexofenadine. *J Clin Pharmacol* **46**:758–767.

Yamaoka K, Tanigawara Y, Nakagawa T, and Uno T (1981) A pharmacokinetic analysis program (multi) for microcomputer. *J Pharmacobiodyn* **4**:879–885.

Yasui-Furukori N, Uno T, Sugawara K, and Tateishi T (2005) Different effects of three transporting inhibitors, verapamil, cimetidine, and probenecid, on fexofenadine pharmacokinetics. *Clin Pharmacol Ther* **77**:17–23.

Address correspondence to: Dr. Yuichi Sugiyama, Department of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. E-mail: sugiyama@mol.f.u-tokyo.ac.jp