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Inhibitory effects of ketoconazole and rifampin on OAT1 and OATP1B1 transport activities: considerations on drug–drug interactions

Min-Koo Choi[a], Qing-Ri Jin[b], Yeong-Lim Choi[c], Sung-Hoon Ahn[d], Myung-Ae Bae[d], and Im-Sook Song[c,*]
[a]*Department of Pharmaceutics, College of Pharmacy, Dankook University, Cheonan 330-714, Korea*
[b]*Department of Pharmaceutics, College of Pharmacy, Seoul National University, Seoul 151-742, Korea*
[c]*Department of Pharmacology and Pharmacogenomics Research Center, Inje University College of Medicine, Busan 614-735, Korea*
[d]*Drug Discovery Platform Technology Team, Korea Research Institute of Chemical Technology, Daejeon, 305-600, Korea*

ABSTRACT: Ketoconazole and rifampin are the most widely used compounds examined in recent drug–drug interaction (DDI) studies, and they have multiple roles in modulating drug metabolizing enzymes and transporters. To determine the underlying mechanisms of DDI, this study was performed to investigate the inhibitory effects of ketoconazole and rifampin on the functions of OAT1 and OATP1B1, and to evaluate the potential of ketoconazole and rifampin for DDI with substrate drugs for these transporters in a clinical setting. Ketoconazole inhibited OATP1B1-mediated transport activity, while rifampin inhibited OAT1 and OATP1B1. Inhibition by rifampin and ketoconazole of the uptake of olmesartan, a substrate for OAT1 and OATP1B1, was evaluated in oocytes overexpressing these transporters. The K_i values for rifampin on OAT1 and OATP1B1-mediated olmesartan uptake were 62.2 and 4.42 μM, respectively, and the K_i value for ketoconazole on OATP1B1-mediated olmesartan uptake was 66.1 μM. As measured plasma concentrations of rifampin and ketoconazole were 7.29 and 6.4–13.3 μM, respectively, the likelihood of an OATP1B1-mediated drug–drug interaction between rifampin and olmesartan is thought to be possible, whereas OAT1 or OATP1B1-mediated DDI between rifampin or ketoconazole and olmesartan appears unlikely in the clinical setting. Copyright © 2011 John Wiley & Sons, Ltd.

Key words: transporter-mediated drug–drug interaction; ketoconazole; rifampin; olmesartan; OAT1; OATP1B1

Introduction

In clinical drug–drug interaction (DDI) studies, multiple doses of an inhibitor are often administered to achieve maximal inhibition. To determine the impact of cytochrome P450 (CYP) 3A inhibition on the clearance of a CYP3A substrate *in vivo*, ketoconazole has been used widely as a strong, reversible inhibitor. A search of the literature and recent new drug applications using a metabolism and transport drug interaction database (University of Washington, Seattle, WA) identified 142 DDI studies on ketoconazole as of October 2008 [1]. Due to the broad overlap of substrate specificities between drug transporters and drug metabolizing enzymes, many inhibitors and inducers can simultaneously affect both drug transporters and CYP enzymes. This should be taken into account when exploring the underlying mechanisms of DDIs [2].

*Correspondence to: Department of Pharmacology and Pharmacogenomics Research Center, Inje University College of Medicine, Gaegum2-dong, Jin-gu, Busan 614-735, Korea.
E-mail: issong@inje.ac.kr

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An important lesson learned from previous animal studies is that transporter inhibition has a much greater impact on the tissue distribution of drugs than it does on systemic exposure [2]. The potential risk of transporter-mediated drug interactions may be underestimated if only plasma concentration is monitored. As HIV can penetrate the brain and cause neurological complications, it is highly desirable that HIV protease inhibitors can penetrate the brain and exert their antiviral activity in the brain [2]. Khaliq *et al*. reported interactions between ketoconazole and the HIV protease inhibitors, ritonavir and saquinavir, in AIDS patients [3]. Co-administration of ketoconazole resulted in significant increases in the plasma and cerebrospinal fluid concentrations of ritonavir and saquinavir, whereas ketoconazole had little effect on plasma protein binding of the drugs [3]. These results suggest that ketoconazole inhibits the functional activity of CYP3A4 and P-gp in humans. The K_i value of ketoconazole to inhibit the metabolism of midazolam in human liver microsomes was determined to be 0.015 μM [4]. The IC_{50} value of ketoconazole to inhibit digoxin transport in Caco-2 cells was determined to be 1.4 μM [5]. As the plasma concentration of ketoconazole achieved with multiple doses of 400 mg q.d. is in the range of 7–11 μg/ml, inhibition of CYP3A4 and P-gp activity by ketoconazole could change the plasma and cerebrospinal fluid concentrations of ritonavir and saquinavir [2,6].

Ketoconazole is also an inhibitor of UGT2B7-catalysed morphine glucuronidation with K_i values of 118 μM for M-3-G and 108 μM for M-6-G [7]. Ketoconazole inhibited UGT1A1-mediated SN38 glucuronidation with K_i value of 3.3 μM [8]. In an *in vivo* DDI study, an FDA guidance document recommended multiple doses of 400 mg ketoconazole as an inhibitor of CYP3A4. Considering the high plasma concentration of ketoconazole when administered as multiple doses of 400 mg ketoconazole, and the multiple inhibitory effects of ketoconazole on the activities of not only CYP3A4 but also UGT and P-gp, drug interactions between ketoconazole and substrate drugs could occur without a clear understanding of the underlying mechanisms. The inhibitory potential of ketoconazole on the transport activity has not been fully evaluated, although 10 and 100 μM ketoconazole was reported to inhibit fexofenadine uptake by 55% and 100%, respectively, in mammalian cells expressing OATP [9].

Rifampin is an activator of PXR nuclear receptor, increasing the transcription of CYP3A4 and P-gp, and, consequently, altering the pharmacokinetics of substrate drugs such as tacrolimus, atorvastatin, verapamil and others [10–13]. Therefore, rifampin is a widely used inducer of CYP3A4 and P-gp in clinical studies investigating DDIs of new molecular entities. However, rifampin is also well known to inhibit OATP1B1 transport activity. Plasma concentrations of atorvastatin and bosentan, representative substrates of OATP1B1, were significantly increased by a single dose of rifampin as a consequence of its inhibitory effect on OATP1B1 [14,15]. Rifampin was also reported to inhibit rat OAT transport activity [16], although the K_i value was not determined.

The present study was performed to investigate the inhibitory effects of ketoconazole and rifampin on the transport activity of OAT1 and OATP1B1 and to evaluate their DDI potentials for OAT1 and OATP1B1 substrate drugs in a clinical setting.

Materials and Methods

Chemicals

[^{14}C]para-Aminohippuric acid (PAH, 1.95 GBq/mmol) and [^{3}H]estrone-3-sulfate (ES, 2.12 TBq/mmol) from Perkin Elmer Inc. (Boston, MA). Olmesartan were purchased from Toronto Research Chemicals (Toronto, ON, Canada). Unlabeled PAH, ES, ketoconazole and rifampin were purchased from Sigma–Aldrich Chemical Co. (St Louis, MO). All other chemicals were reagent grade and all solvents were HPLC grade.

Inhibitory effects of ketoconazole and rifampin on OAT1 and OATP1B1 transport activity

Plasmid DNAs containing OAT1 and OATP1B1 were constructed and confirmed by full sequencing with forward and reverse sequencing primers to ensure that no unwanted mutations occurred, as previously described [17,18].

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cRNA synthesis and uptake experiments were performed as described previously [17]. Briefly, capped cRNAs were synthesized *in vitro* using T7 RNA polymerase with linear plasmid DNA. Defolliculated oocytes were injected with 50 ng of the capped cRNA and incubated at 18°C in Barth's solution [88 mM NaCl, 1 mM KCl, 0.33 mM $Ca(NO_3)_2$, 0.4 mM $CaCl_2$, 0.8 mM $MgSO_2$, 2.4 mM $NaHCO_3$, 10 mM HEPES (pH 7.4)] containing 50 μg/ml gentamicin and 2.5 mM pyruvate. After incubation for 2 days, uptake experiments were performed at room temperature in ND96 solution [96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, (pH 7.4)] containing [^{14}C]PAH and [^{3}H]ES, representative substrates for OAT1 and OATP1B1, respectively. After washing five times, the oocytes were solubilized with 10% SDS, and the radioactivity in the oocytes was analysed. When examined the inhibitory effect of ketoconazole and rifampin on the transport activity, the uptake of [^{14}C]PAH for OAT1 and [^{3}H]ES for OATP1B1 was measured in the presence of 0.1–200 μM ketoconazole and rifampin, respectively.

Estimation of K_i values for ketoconazole and rifampin on the OAT1 and OATP1B1-mediated olmesartan uptake

To evaluate the inhibitory effects of ketoconazole and rifampin on the OAT1 and OATP1B1 mediated olmesartan transport function, the inhibition experiments were initiated by replacing the ND96 solution with olmesartan (0.5, 2, 10 μM) in the presence of various concentrations of ketoconazole and rifampin (0.1–200 μM) after expressing OAT1 and OATP1B1 in oocytes. After incubation for 30 min, the reactions were terminated by the addition of ice-cold ND96 solution. After washing five times, the oocytes were sonicated in 75% acetonitrile, followed by centrifugation. The supernatant was evaporated, and the residue was reconstituted in 100 μl of mobile phase solution [acetonitrile: DDW containing 0.1% formic acid = 60: 40 (v/v)].

LC/MS/MS analysis

The concentration of olmesartan was quantified by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), as described previously with a slight modification [19]. After centrifugation, a 10 μl aliquot was injected into a Qtrap 4000 MS/MS system (Applied Biosystems, Foster City, CA) equipped with Agilent 1100 series HPLC system (Agilent). The compounds were separated on a reversed-phase column Xbridge^{TM} C_{18} column (2.1 × 50 mm, 2.5 μm, Waters, Milford, MA) at a flow rate of 0.2 ml/min. The retention time for olmesartan and propranolol (internal standard) was 1.38 min and 0.94 min, respectively. Quantitation was performed by multiple reaction monitoring of the deprotonated precursor ion and the related product ion for olmesartan using the internal standard method with peak area ratios and a weighting factor. The mass transition used for olmesartan and propranolol (internal standard) were *m/z* 447.3 → 207.0 and 260.0 → 116.0, respectively. The analytical data were processed by Analyst software (ver. 1.41, Applied Biosystems). For all compounds, calibration studies indicated that the detector response was linear over 0.5–100 ng/ml for olmesartan below 10.9% of interday and intraday coefficients of variation. Accuracy was ranged from 92.7–105.4%.

Results

Inhibitory effects of ketoconazole and rifampin on the OAT1 and OATP1B1 transport activity

To confirm the functionality of a *Xenopus laevis* oocyte system expressing OAT1 and OATP1B1 transporters, uptake of [^{14}C]PAH, a representative substrate for OAT1, and [^{3}H]ES, a representative substrate for OATP1B1 were measured. The uptake of 1 μM [^{14}C]PAH and 20 nM [^{3}H]estrone-3-sulfate showed enhancement by 18.7-fold and 238-fold, respectively, in oocytes expressing OAT1 and OATP1B1 transporters, compared with water-injected controls (0.264 ± 0.097 fmol/oocyte in control vs 62.9 ± 8.5 fmol/oocyte in OAT1 for [^{14}C]PAH uptake; 42.7 ± 9.7 fmol/oocyte in control vs 799 ± 63 fmol/oocyte in OATP1B1 for [^{3}H]ES uptake). These results confirmed the functionality of this oocyte expression system.

To investigate the inhibitory effects of ketoconazole and rifampin on OAT1 and OATP1B1

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transporters, the uptake of [^{14}C]PAH and [^{3}H]ES by oocytes expressing OAT1 and OATP1B1, was measured in the presence of 0.1–200 μM ketoconazole or rifampin. Rifampin inhibited OAT1-mediated PAH uptake and OATP1B1-mediated ES uptake in a concentration dependent manner (Figure 1). When the relevant data were fitted to an inhibitory effect model ($v = V_{max}(1[I]/(IC_{50} + [I]))$ using Winnonlin 5.2 software, IC_{50} values for rifampin on OAT1 and OATP1B1 were estimated to be 79.1 and 5.16 μM, respectively. Similar to rifampin, ketoconazole also inhibited OAT1-mediated PAH uptake and OATP1B1-mediated ES uptake in a concentration dependent manner, but to a lesser extent (Figure 2). IC_{50} values for ketoconazole on OAT1 and OATP1B1 were estimated to be 319 and 43.4 μM, respectively.

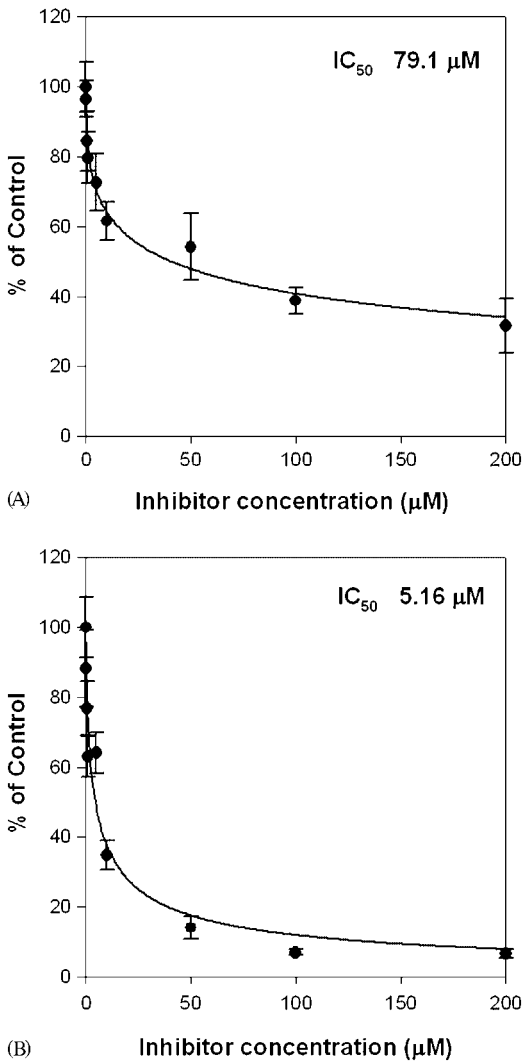

Figure 1. Inhibitory effect of rifampin on the transport activities of OAT1 (A) and OATP1B1 (B). Inhibitory effect of rifampin (0.1–200 μM) on the uptake of 1 μM [^{14}C]para-aminohippuric acid (PAH) and 0.1 μM [^{3}H]estrone-3-sulfate (ES) was measured in oocytes expressing OAT1 and OATP1B1, respectively. The black bar represents the mean ± SD of three independent experiments. Data were fitted to an inhibitory effect E_{max} model [i.e. $v = E_{max} - E_{max} \times C/(IC_{50}+C)$], and the IC_{50} value was calculated

Estimation of K_i values for rifampin and ketoconazole on OAT1 and OATP1B1-mediated olmesartan uptake

The study next examined the inhibitory effects of rifampin and ketoconazole (0.1–200 μM) on the uptake of olmesartan in oocytes expressing OAT1 and OATP1B1 transporters. Figure 3A exhibited the inhibition profile of rifampin for OAT1-mediated olmesartan uptake at 0.5, 1 and 10 μM olmesartan. These data were transformed into a Dixon plot, and the K_i was calculated (Figure 3B). The K_i value for rifampin inhibition of OAT1-mediated olmesartan uptake was 62.2 μM. A replot of the Dixon slopes produced a straight line converging on the origin, suggesting competitive inhibition (Figure 3C).

As olmesartan is also a substrate for OATP1B1, the effects of rifampin on OATP1B1-mediated olmesartan uptake were investigated. The inhibition profile of rifampin on OATP1B1-mediated olemsartan uptake is shown in Figure 4A. Again, inhibition data for three different olmesartan concentrations (0.5, 1 and 10 μM) were transformed into a Dixon plot, enabling calculation of the K_i value (Figure 4B). The K_i value of rifampin on OATP1B1-mediated olmesartan transport was 4.42 μM. A replot of the Dixon slope vs $1/[I]$ indicated competitive inhibition by rifampin (Figure 4C).

A similar approach was used to examine the inhibition by ketoconazole of OATP1B1-mediated olmesartan uptake. The inhibition profile of ketoconazole on OATP1B1-mediated olmesartan uptake was shown in Figure 5A. The K_i value for ketoconazole, determined as described above for rifampin, was 66.1 μM (Figure 5B). The replot of the Dixon slope vs $1/[I]$

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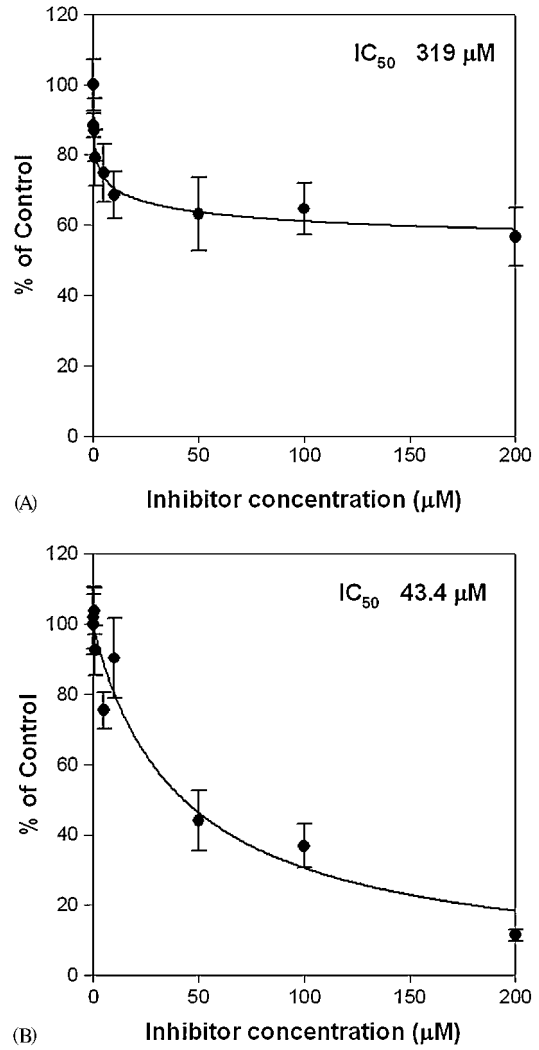

Figure 2. Inhibitory effect of ketoconazole on the transport activities of OAT1 (A) and OATP1B1 (B). Inhibitory effect of ketoconazole (0.1–200 μM) on the uptake of 1 μM [^{14}C]PAH and 0.1 μM [^{3}H]ES was measured in oocytes expressing OAT1 and OATP1B1, respectively. The black bar represents the mean ± SD of three independent experiments. Data were fitted to an inhibitory effect E_{max} model [i.e. $v = E_{max} - E_{max} \times C/(IC_{50}+C)$], and the IC_{50} value was calculated

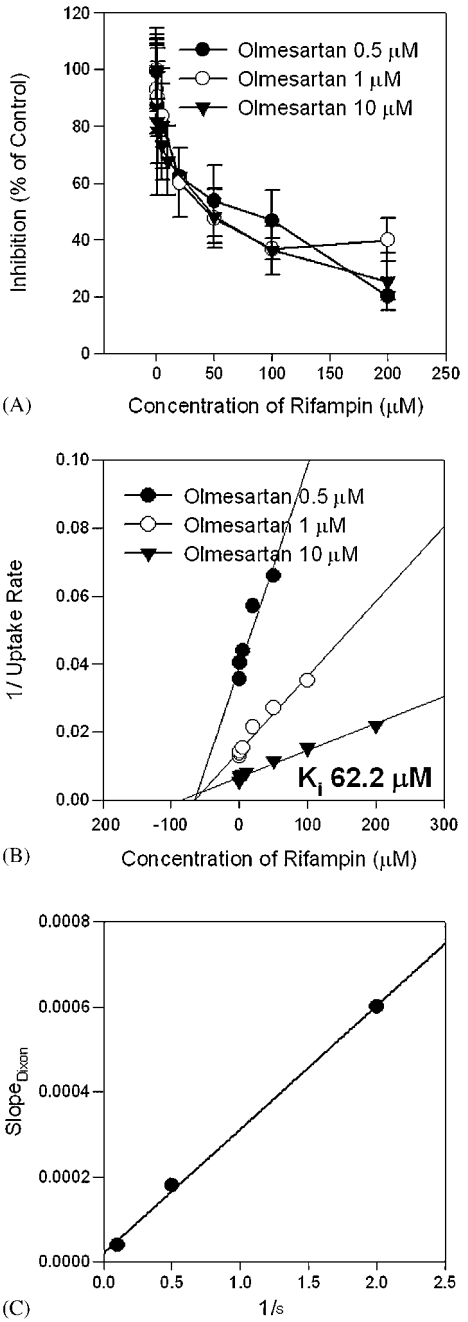

Figure 3. (A) Effect of rifampin (0.1−200 μM) on the kinetics of OAT1-mediated uptake of 0.5, 1 and 10 μM olmesartan (●, ○ and ▼, respectively) in oocytes expressing OAT1. (B) The OAT1-mediated uptake data at 0.5, 1 and 10 μM olmesartan (●, ○ and ▼, respectively) in the presence of various concentration of rifampin (0.1−200 μM) were transformed into a Dixon plot, and the K_i values of rifampin for OAT1-mediated olmesartan uptake were calculated. (C) Replot of the slopes of Dixon plot (slopes vs 1/[*S*]) was shown. *S* indicates the concentration of rifampin (μM)

also indicated competitive inhibition by ketoconazole of OATP1B1-mediated olmesartan uptake (Figrue 5C). However, ketoconazole did not inhibit the OAT1-mediated olmesartan uptake (Figure 6A) and, consequently, the K_i value could not calculated (Figure 6B).

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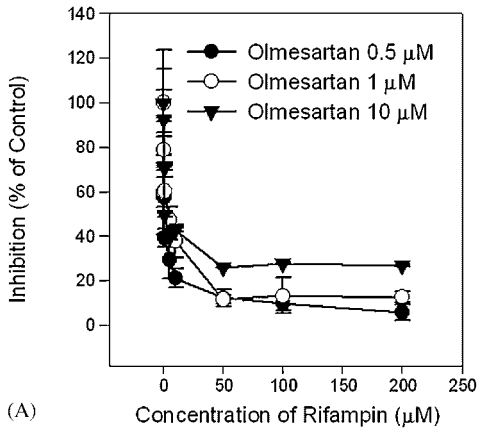
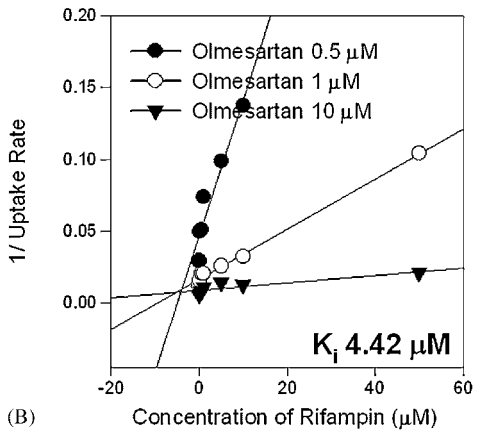




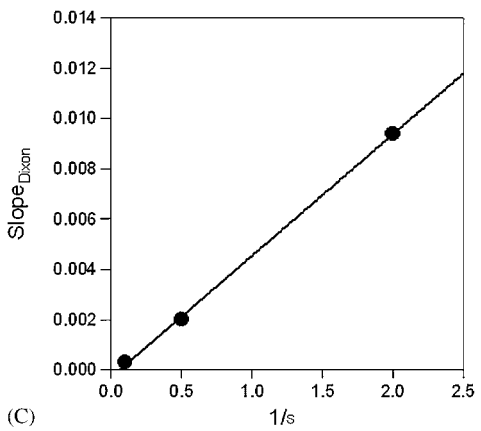
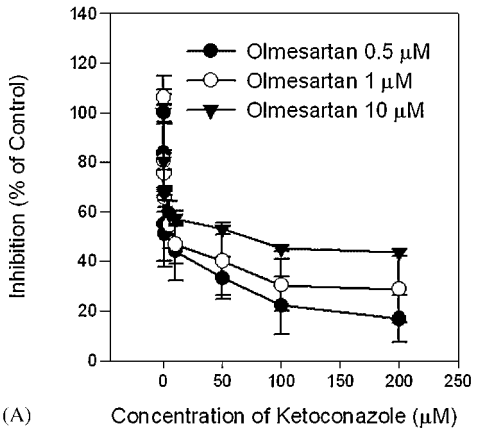
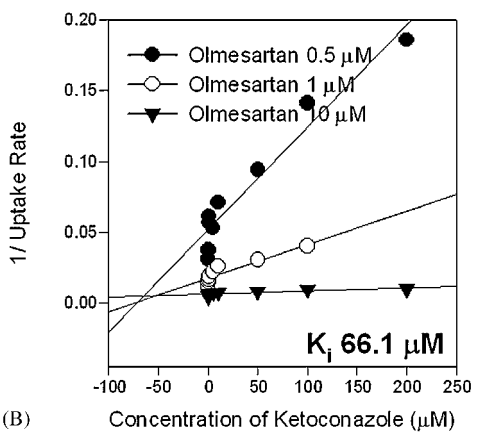


Figure 4. (A) Effect of rifampin (0.1−200 μM) on the kinetics of OATP1B1-mediated uptake of 0.5, 1 and 10 μM olmesartan (●, ○ and ▼, respectively) in oocytes expressing OATP1B1. (B) The OATP1B1-mediated uptake data at 0.5, 1 and 10 μM olmesartan (●, ○ and ▼, respectively) in the presence of various concentration of rifampin (0.1−200 μM) were transformed into a Dixon plot, and the K_i values of rifampin for OATP1B1-mediated olmesartan uptake were calculated. (C) Replot of the slopes of Dixon plot (slopes vs 1/[*S*]) was shown. *S* indicates the concentration of rifampin (μM)





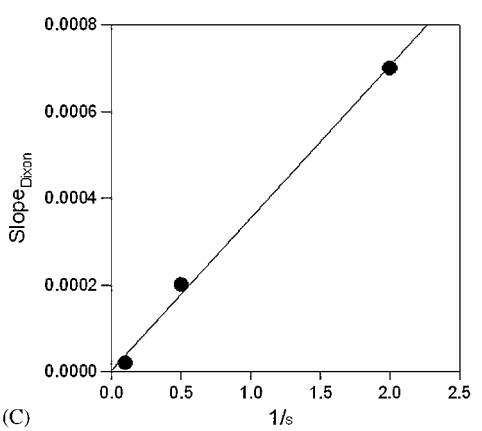


Figure 5. (A) Effect of ketoconazole (0.1−200 μM) on the kinetics of OATP1B1-mediated uptake of 0.5, 1 and 10 μM olmesartan (●, ○ and ▼, respectively) in oocytes expressing OATP1B1. (B) The OATP1B1-mediated uptake data at 0.5, 1 and 10 μM olmesartan (●, ○ and ▼, respectively) in the presence of various concentration of ketoconazole (0.1−200 μM) were transformed into a Dixon plot, and the K_i values of ketoconazole for OATP1B1-mediated olmesartan uptake were calculated. (C) Replot of the slopes of Dixon plot (slopes vs 1/[*S*]) was shown. *S* indicates the concentration of ketoconazole (μM)

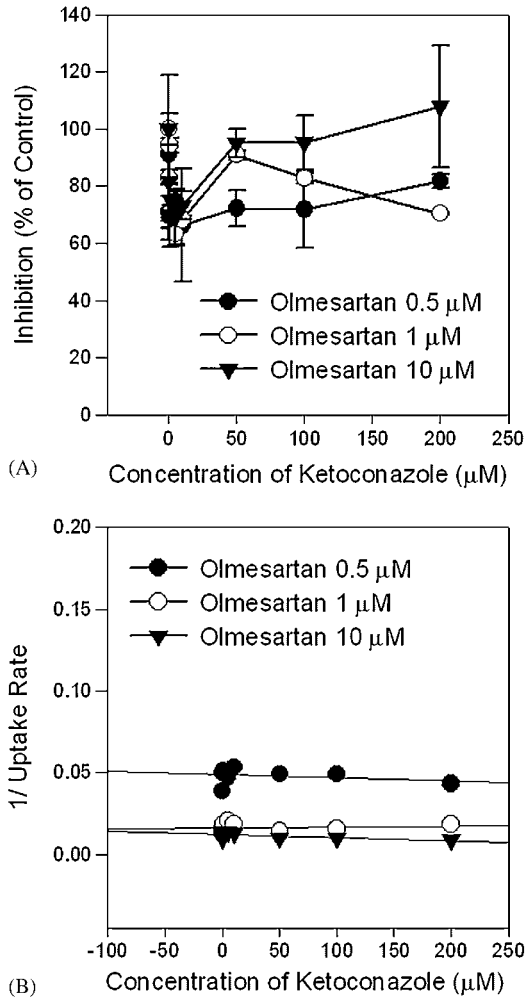


Figure 6. (A) Effect of ketoconazole (0.1−200 μM) on the kinetics of OAT1-mediated uptake of 0.5, 1 and 10 μM olmesartan (●, ○ and ▼, respectively) in oocytes expressing OAT1. (B) The OAT1-mediated uptake data at 0.5, 1 and 10 μM olmesartan (●, ○ and ▼, respectively) in the presence of various concentration of ketoconazole (0.1−200 μM) were transformed into a Dixon plot

Discussion

The recent trend toward the use of polypills, which combine drugs with different mechanisms of action, necessitates careful consideration of DDIs between the combined medicines. DDIs between inhibitors and substrates of metabolizing enzymes or transporters can cause serious adverse reactions. Therefore, prediction and evaluation of DDI potential is important in the clinic and in the drug development process.

Ketoconazole and rifampin are the most widely used inhibitory drugs in DDI studies. In previous studies, rifampin inhibited OATP1B1 transport activity with K_i values of 1.5–17 μM [20,21], which is similar to the IC_{50} value (5.16 μM) calculated in the present study. Rifampin was also reported to inhibit rat OAT2 transport activity [16], but the IC_{50} value for OAT1 was not determined. This study is the first report showing the inhibitory effect of rifampin and ketoconazole on OAT1-mediated transport activity. That is, the IC_{50} value of rifampin on OAT1 transport activity was 79.1 μM, indicating that the inhibitory potential of rifampin is much higher against OATP1B1 than against OAT1. Our study also showed that ketoconazole inhibited OATP1B1 and OAT1 transport activity with IC_{50} values of 43.4 and 319 μM, respectively.

To evaluate the inhibitory potential of ketoconazole and rifampin on OAT1 and OATP1B1 transport activity, their effects using olmesartan as a substrate were examined. Olmesartan, a newly developed angiotensin II receptor antagonist for the treatment of hypertension, is orally administered in the prodrug form, olmesartan medoxomil [22]. After intravenous administration of olmesartan (16 mg), the amount excreted into urine was 35.8 ± 5.9% of dose and the hepatic clearance accounted for about 60% of total clearance in healthy male subject, without significant metabolism by CYPs [23–25]. Therefore, olmesartan is unlikely to interact with drugs that inhibit, induce or are metabolized by CYPs. The permeability of olmesartan was reported to be about 1.0×10^{-6} cm/s, which is similar to that of Lucifer yellow, in Caco-2 cells [26]. Moreover, active intrinsic clearance of olmesartan was 6.8-fold greater than passive diffusion in OATP1B1-expressing HEK293 cells. These results, taken together, suggest that active pathways of olmesartan dominate passive diffusion and drug transporters, such as OAT1, OAT3, OATP1B1, OATP1B3 and MRP2, play a significant role in the pharmacokinetics of olmesartan [27]. Among them, OAT1 and OAT3 are expected to play important roles in the renal elimination of olmesartan, with relative contributions of 7.05%–14.2% and 85.8%–93% in human kidney slices, respectively. Likely, OATP1B1 and OATP1B3 play crucial roles in the hepatobiliary excretion of this

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drug, with relative contributions in human hepatocytes of 44.3%–62.2% and 37.8%–55.7%, respectively [27]. The C_{max} and *AUC* values of olmesartan tended to be higher while *CL/F* value was significantly lower in subjects with OATP1B1*15/*15 variation, compared with OATP1B1 wild type, suggesting the importance of OATP1B1 in the pharmacokinetics of olmesartan [28]. Moreover, OAT1 and OATP1B1 showed higher affinities for olmesartan than OAT3 and OATP1B3 [27]. Therefore, we firstly investigated the inhibitory effects of ketoconazole and rifampin on the transport activity of OAT1 and OATP1B1 and to evaluate their DDI potentials for OAT1 and OATP1B1 substrate drug, olmesartan, in a clinical situation.

In the case in which a drug is mainly eliminated by a renal and/or biliary route via drug transporters, that are competitively inhibited by concomitantly administered drugs, changes in the clearance and *AUC* of given drugs can be expressed as $CL/CL_i = AUC_i/AUC = 1 + [I]/K_i$ [29]. Although olmesartan, pravastatin, rosuvastatin and pitavastatin are mainly eliminated by drug transporters such as OATP1B1, DDI between OATP1B1 inhibitors (i.e. rifampin, gemfibrozil, and cyclosporine A) and substrates (i.e. olmesartan, pravastatin, rosuvastatin and pitavastatin) could be shown to depend on the contribution of the responsible transporters such as OATP1B1 to the overall pharmacokinetics of substrates and their affinities to the transporters. Thus, careful consideration on the intrinsic disposition pathway as well as the affinities of the substrates to the target transporter should be needed.

To avoid false-negative prediction of DDI, the possibility of a drug interaction can be estimated from the $C_{max,unbound}/K_i$ ratio. The C_{max} values of rifampin administered as a single dose or as a repeated dose of 600 mg for 5 consecutive days were 9.48 μM and 7.29 μM, respectively, and plasma protein binding was 89% [30,31]. Therefore, the $C_{max,unbound}$ value of rifampin was calculated as 1.04 μM and 0.80 μM, respectively. The C_{max} value for ketoconazole administered at a dose of 400 mg was reported to be 6.4–13.3 μM and plasma protein binding was 84–99% [32,33]. Therefore, the $C_{max,unbound}$ value of ketoconazole was calculated as 0.064–2.13 μM and 0.80 μM, respectively. Given the K_i values for rifampin inhibition of OAT1 and OATP1B1-mediated olmesartan transport (62.2 and 4.42 μM, respectively), the likelihoods of DDI ($C_{max,unbound}/K_i$ ratio) were 0.017 and 0.24, respectively. This indicates a remote probability of an OAT1-mediated interaction between olmesartan and rifampin, but a low possibility of OATP1B1-mediated interaction. Similarly, given the 66.1 μM K_i value for ketoconazole inhibition of OATP1B1-mediated olmesartan transport, the likelihood of DDI was 0.00097–0.032, indicating a remote probability.

In conclusion, the inhibitory effects of ketoconazole and rifampin on OAT1 and OATP1B1 transport activities were investigated, to explore the underlying mechanism of DDI. While both ketoconazole and rifampin have inhibitory effects on OAT1 and OATP1B1-mediated transport, only the effect of rifampin on OATP1B1 appears a low DDI possibility to occur under clinical conditions.

Acknowledgements

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