Effect of coadministration of single and multiple doses of rifampicin on the pharmacokinetics of fexofenadine enantiomers in healthy subjects

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Abbreviations:

AUC, area under the curve; CCK-8, cholecystokinin octapeptide; DDI, drug-drug interaction; HPLC, high pressure liquid chromatography; LC–MS/MS, liquid chromatography–tandem mass spectrometry; MATE, multidrug and toxic compound extrusion; OATP, organic anion transporting polypeptide; OAT, organic anion transporter
Abstract

Effect of rifampicin on the pharmacokinetics of fexofenadine enantiomers was examined in healthy subjects who received fexofenadine alone or with single or multiple doses of rifampicin (600 mg). A single coadministered dose of rifampicin significantly decreased the CL\textsubscript{tot}/F and CL\textsubscript{r} of $S$- and $R$-fexofenadine by 76% and 62%, and 73% and 62%, respectively. Even after multiple doses, rifampicin significantly decreased these parameters although the effect on the CL\textsubscript{tot}/F was slightly blunted. Multiple doses of rifampicin abolished the difference in the CL\textsubscript{tot}/F of fexofenadine enantiomers, whereas the stereoselectivity in the CL\textsubscript{r} persisted. Rifampicin inhibited the uptake of fexofenadine enantiomers by human hepatocytes via OATP1B3 and its basal-to-apical transport in Caco-2 cells, but not OAT3- or MATE1-mediated transport. The plasma unbound fraction of $S$-fexofenadine was 1.8 times higher than that of $R$-fexofenadine. The rifampicin-sensitive uptake by hepatocytes was 1.6 times higher for $R$-fexofenadine, whereas the transport activities by OATP1B3, OAT3, MATE1 or P-glycoprotein were identical for both enantiomers. $S$-fexofenadine is a more potent human histamine H\textsubscript{1} receptor antagonist than $R$-fexofenadine. In conclusion, rifampicin has multiple interaction sites with fexofenadine, all of which contribute to increasing the AUC of fexofenadine, when they are given simultaneously, to surpass the effect of the induction of P-glycoprotein elicited by multiple doses.
Introduction

Drug–drug interactions (DDIs) involving metabolism and/or excretion processes alter the pharmacokinetics of victim drugs, and consequently their pharmacological/adverse effects. They are one of the leading causes of withdrawal of new drugs from the market. Recent progress in the study of membrane transport has expanded our understanding of the mechanisms underlying pharmacokinetic DDIs involving transporters (Giacomini et al., 2010; Yoshida et al., in press).

Fexofenadine, a selective and nonsedative histamine H1 receptor antagonist, is one of the most frequently used treatments for seasonal allergic rhinitis and chronic urticaria. As fexofenadine is hardly metabolized by cytochrome P450, transporters play an indispensable role in fexofenadine pharmacokinetics. Actually, it is a substrate of OATP1A2, OATP1B3, OATP2B1, OAT3, MATE1, BSEP, MRP2, MRP3, and P-glycoprotein (P-gp) (Cvetkovic et al., 1999; Nozawa et al., 2004; Shimizu et al., 2005; Tahara et al., 2005; Tahara et al., 2006; Matsushima et al., 2008a; Tian et al., 2008; Matsushima et al., 2009). A number of drug interaction studies have reported for fexofenadine to elucidate the impact of inhibition of P-gp-mediated efflux or the influx transporter in the small intestine by erythromycin, itraconazole, ketoconazole, ritonavir and verapamil (Dresser et al., 2002; Tachibana et al., 2009) and inhibition of OAT3 and MATE1 in the urinary excretion by probenecid and cimetidine, respectively (Yasui-Furukori et al., 2005; Tahara et al., 2006; Matsushima et al., 2009). However, the impact of inhibition of hepatic elimination on fexofenadine pharmacokinetics remains to be evaluated although hepatic elimination is the major elimination pathway of fexofenadine from the systemic circulation (Lappin et al., 2010).

We suggested that OATP1B3, a multispecific organic anion transporter predominantly expressed in the liver, mediate the hepatic uptake of fexofenadine based on in vitro experiments (Shimizu et al., 2005). In addition, a pharmacogenomic study focused on the genotypes of OATP1B1, another hepatic uptake transporter, suggests that OATP1B1 also mediate the hepatic uptake of fexofenadine (Niemi et al., 2005). Rifampicin, which is used to treat tuberculous meningitis, is well known to induce drug-metabolizing enzymes and transporters through the activation of the nuclear
receptor pregnane X receptor, and consequently to lower the systemic exposure of victim drugs. Indeed, Hamman et al. reported that multiple doses of rifampicin markedly reduced the AUC of fexofenadine in healthy subjects (Hamman et al., 2001). On the other hand, simultaneous administration of rifampicin increases the systemic exposure of OATP substrates such as atorvastatin, glibenclamide, and pravastatin, by inhibiting their hepatic uptake by OATP1B1 (Lau et al., 2007; Zheng et al., 2009; Maeda et al., 2011). As the Ki value of rifampicin for OATP1B3 is similar to that for OATP1B1 (Matsushima et al., 2008b), rifampicin will inhibit OATP1B3 and consequently increase the AUC of its substrate drugs when both drugs are given simultaneously. In the previous study conducted by Hamman et al., as the final dose of rifampicin was given at bedtime, inhibition of OATPs by rifampicin became negligible when fexofenadine was given to the subjects. Furthermore, fexofenadine is administered therapeutically as a racemic mixture of S- and R-enantiomers. The plasma concentration of S-fexofenadine in humans is about 1.5 times lower than that of the corresponding R-fexofenadine, although the underlying mechanism of this difference remains unknown (Robbins et al., 1998; Miura et al., 2007). Notably, the impact of DDI on the CL\textsubscript{tot}/F of fexofenadine is stereoselective: a single dose of itraconazole or multiple doses of carbamazepine blunted the stereoselective pharmacokinetics of fexofenadine enantiomers (Tateishi et al., 2008; Sakugawa et al., 2009; Akamine et al., 2012).

We designed this clinical study to investigate the effect of coadministered rifampicin on the pharmacokinetics of fexofenadine enantiomers in healthy subjects who receive single and multiple doses of rifampicin, and conducted \textit{in vitro} studies to support the clinical data.
Materials and Methods

Materials

\[^{3}\text{H}\] Estrone-3-sulfate (54.3 Ci/mmol) and \[^{3}\text{H}\] pyridinyl-5-pyrilamine (\[^{3}\text{H}\] mepyramine, 20 Ci/mmol) were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA), and \[^{3}\text{H}\] CCK-8 (97.5 Ci/mmol) from Amersham Biosciences UK, Ltd. (Little Chalfont, Buckinghamshire, UK). \(^R\) and \(^S\)-fexofenadine were purchased from Toronto Research Chemicals Inc. (Ontario, Canada), and cetirizine was purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals used were commercially available and of analytical grade. Cryopreserved human hepatocytes (TDH and 03-013) were purchased from In Vitro Technologies Inc. (Baltimore, MD) and Life Technologies (Durham, NC), respectively, and kept in liquid nitrogen until use.

Clinical study in healthy subjects

Clinical study was conducted in Hirosaki University. Twelve healthy Japanese volunteers (eight men and four women) were enrolled in this study after giving informed written consent. Each subject was physically normal according to clinical examination and routine laboratory testing and had no history of significant medical illness or hypersensitivity to any drugs. Their mean values for age and body weight were 23.8±5.6 years (range, 20–41 years) and 55.7±7.8 kg (range, 42–70 kg), respectively. This study was approved by the Ethics Committee of Hirosaki University School of Medicine.

This randomized open-label study consisted of two (control and 6-day treatment) phases and four study days. In the control phase, the volunteers received 60 mg fexofenadine hydrochloride (Allegra\textsuperscript{®}, Aventis Pharma Ltd, Tokyo, Japan) at 0800 h after an overnight fast. In the treatment phase, the volunteers received 600 mg rifampicin (Rifadin\textsuperscript{®}, Daiichi-Sankyo Pharmaceutical, Tokyo, Japan) at 0800 h for 6 days. On days 1, 3, and 6, each subject was simultaneously administered 60 mg fexofenadine hydrochloride with rifampicin after an overnight fast. The order of the two phases was randomly assigned for each volunteer. Six volunteers started the control phase first and their treatment phase was started more than 24 h after the last blood sampling of the control phase. The remaining
subjects started the treatment phase first, and their control phase was started more than 2 weeks after the last sampling. The volunteers did not take any other medication or fruit juice for at least 7 days before each phase, and no food or beverages were allowed until 3 h after fexofenadine administration.

Blood samples (10 mL each) were drawn before and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after fexofenadine administration. Time points for blood sampling follow those previously reported (Yasui-Furukori et al., 2005). A spot of urine was collected as a blank sample before fexofenadine administration and thereafter the urine was collected until 24 h after administration. Plasma and urine concentrations of fexofenadine were determined by the HPLC method developed in our laboratory (Miura et al., 2007). Plasma and urine samples from the treatment phase did not have any interfering peak for the assay, and the plasma and urine samples before fexofenadine administration had no detectable fexofenadine peak.

The maximum plasma concentration (Cmax) and the time to reach Cmax (tmax) were determined directly from the observed data. The elimination rate constant (λz) was obtained by linear regression analysis by use of at least three sampling points of the terminal log-linear declining phase to the last measurable concentration. The elimination half-life (t½) was calculated as 0.693 divided by λz. The area under the plasma concentration–time curve from time zero to infinity (AUC 0–∞) was calculated using AUC (0–12) + Clast/λz, where Clast was the plasma drug concentration at 12 h. The apparent oral clearance (CLtot/F), the apparent volume of distribution (Vd/F), and CLr were obtained using the following equations:

\[
\text{CL_{tot}/F} = \frac{\text{Dose}}{\text{AUC}_{0-\infty}}
\]

\[
\text{CL}_r = \frac{\text{Ae}_{0-24}}{\text{AUC}_{0-24}}.
\]

**Determination of the uptake of fexofenadine by cDNA transfected cells**

OATP1B1-, OATP1B3-, OAT3-, MATE1-, and MATE2-K-expressing HEK293 cell lines were constructed previously (Deguchi et al., 2004; Hirano et al., 2004; Matsushima et al., 2009). The transport study was performed as described previously (Hirano et al., 2004). Briefly, the cells were
incubated with Krebs–Henseleit buffer (118 mM NaCl, 23.8 mM NaHCO₃, 4.83 mM KCl, 0.96 mM KH₂PO₄, 1.20 mM MgSO₄, 12.5 mM HEPES, 5.0 mM glucose, and 1.53 mM CaCl₂ adjusted to pH 7.4) containing radiolabeled or nonradiolabeled compounds at 37 °C in the presence or absence of inhibitors. Uptake was terminated by the addition of ice-cold buffer after removal of the incubation buffer. The fexofenadine concentration associated with the cell specimens was determined by LC–MS/MS analysis.

**Determination of the uptake of fexofenadine by cryopreserved human hepatocytes**

The uptake of fexofenadine was determined in cryopreserved human hepatocytes as reported previously, with minor modifications (Shitara et al., 2003). Briefly, hepatocytes were suspended in Krebs–Henseleit buffer to give a cell density of 1.0 × 10⁶ viable cells/mL for the uptake study. After preincubation at 37 °C for 3 min, uptake was initiated by adding labeled and unlabeled substrates to the cell suspension, and terminated at the designated time by separating the cells from the substrate solution using a layer of oil mixture (density 1.015, a mixture of silicone oil and mineral oil; Sigma-Aldrich) and centrifugation for 10 s in a tabletop centrifuge (10,000 g; Beckman Microfuge E, Beckman Coulter, Inc.). During this process, the hepatocytes passed through the oil layer into the lower layer of 2N NaOH for estrone-3-sulfate and CCK-8 or 5 M ammonium acetate for fexofenadine. The amount of substrate in each compartment was measured with a liquid scintillation counter or LC–MS/MS.

**Determination of transcellular transport of fexofenadine across Caco-2 cells**

Briefly, Caco-2 cells were cultured on Transwell membrane inserts (6.5 mm diameter, 0.4 μm pore size; Corning Costar) for 25–30 days. Cells were first washed with Krebs–Henseleit buffer at 37 °C. Subsequently, fexofenadine and inhibitors (1 mL) were added to the basolateral compartment to start the experiments. Simultaneously, inhibitors were added to the apical compartment (250 μL). After 90 min incubation, 100 μL of the buffer in the apical compartment was collected.
Quantification of fexofenadine and celiprolol by LC–MS

Concentrations of fexofenadine and celiprolol were measured on an AB SCIEX QTRAP 5500 mass spectrometer (Applied Biosystems, Foster City, CA) equipped with a Prominence HPLC system (Shimadzu) operated in the positive electrospray ionization mode. Chromatographic separation was achieved on an Inertsil ODS-4 column (30 mm × 2.1 mm ID, 3 μm; GL Science, Tokyo, Japan) in binary gradient mode at a flow rate of 0.4 mL/min. The mobile phase comprised 0.1% formic acid and acetonitrile. The acetonitrile concentration was initially 5% or 20%; it was then linearly increased to 80% or 90% over 1 or 2 min, and maintained for a further 0.5 or 1.2 min. Finally, the column was reequilibrated with acetonitrile at a concentration of 5% or 20% for 1 min. Mass-to-charge transition were 502.4–466.0 for fexofenadine, and 380.1–251.2 for celiprolol, respectively.

Membrane preparation and binding assay

Human histamine H1 receptor (D28481.1) was cloned from a human cerebral cortex cDNA library. Transient transfection of HEK293 cells was performed using pcDNA3.1(+) (Invitrogen) and FuGENE HD (Roche) according to the manufacturers’ protocols. The [3H]-pyrilamine binding assay was performed as described previously (Ohuchi et al., 1998; Gillard and Chatelain, 2006). Briefly, membranes prepared from the cDNA transfectants (25 μg of protein) were incubated for 60 min at 37 °C in 0.5 mL of 50 mM Na2/K phosphate buffer (pH 7.4) containing [3H]-mepyramine (3 nM) and competing drugs. Samples were rapidly filtered by vacuum through 0.1% polyethyleneimine-pretreated 24 mm glass fiber filters (Whatman GF/C), and the filters were washed four times with 2 mL ice-cold Na2/K phosphate buffer. Nonspecific binding was defined as the amount of [3H]-pyrilamine bound in the presence of 10 μM cetirizine. Radioactivity retained on the filter was measured in a liquid scintillation counter (LS 6000SE; Beckman Instruments, Fullerton, CA). Data were analyzed according to the model proposed by Motulsky and Mahan (Motulsky and Mahan, 1984).
Statistical analysis

The data are presented as means±standard error (SE). Student’s two-tailed nonpaired t test and a one-way ANOVA followed by Dunnett’s post hoc test were used to identify significant differences between groups where appropriate. *P*<0.05 was considered to be significant.
Results

Effect of single and multiple doses of rifampin on the pharmacokinetics of fexofenadine

No clinically undesirable signs or symptoms attributable to the administration of fexofenadine and rifampicin were recognized during the study. All subjects completed the study successfully according to the protocol.

Plasma concentrations of fexofenadine enantiomers in healthy subjects who received fexofenadine alone or with single or multiple doses of rifampicin are shown in Figure 1. The plasma concentration of fexofenadine enantiomers at last sampling point (24 after the administration) was below the lower limit of quantification. The pharmacokinetic parameters of fexofenadine enantiomers are summarized in Table 1. There was no sex difference in the pharmacokinetic parameters of fexofenadine enantiomers (data not shown). A single dose of rifampicin significantly decreased the CL_{tot}/F and CL_{r} by 77% and 62%, respectively, for S-fexofenadine, and by 73% and 61%, respectively, for R-fexofenadine. Rifampicin also significantly decreased the CL_{tot}/F and CL_{r} for S-fexofenadine by 66% and 54%, respectively and for R-fexofenadine by 57% and 54%, respectively, after multiple doses. Individual data is shown in Figure 2. The CL_{tot}/F and CL_{r} of S-fexofenadine were 1.5 and 1.7 times larger than those of R-fexofenadine (Table 1). Multiple doses of rifampicin abolished the difference in the CL_{tot}/F of fexofenadine enantiomers, whereas the stereoselectivity in the CL_{r} persisted both after single and multiple doses. The unbound fraction of fexofenadine in human plasma, measured using equilibrium dialysis, was stereoselective: 1.8 times higher for S-fexofenadine than for R-fexofenadine (0.235±0.019 versus 0.134±0.005 [mean ± SE]).

Effect of rifampicin on the uptake of R- and S-fexofenadine by OATP1B3

The uptake of fexofenadine enantiomers was determined in two batches of cryopreserved human hepatocytes (lot TDH and lot 03-013), which were characterized by the difference in the uptake of an OATP1B3 reference substrate, CCK-8 (Figure 3A). The uptake of both R- and S-fexofenadine was higher in lot TDH than in lot 03-013. Rifampicin significantly inhibited the uptake of fexofenadine only in lot TDH. The level of uptake of fexofenadine that remained in the presence of
rifampicin was similar in the two lots. The rifampicin-sensitive uptake of \( R \)-fexofenadine was 1.6 times higher than that of \( S \)-fexofenadine.

The effect of rifampicin on the uptake of fexofenadine enantiomers was determined in HEK293 cells expressing OATP1B3 (OATP1B3-HEK) (Figure 3B). The uptake of fexofenadine enantiomers was significantly greater in OATP1B3-HEK than in control mock vector-transfected cells. Rifampicin inhibits OATP1B3-mediated uptake of fexofenadine enantiomers, and the specific uptake by OATP1B3 was abolished at 2 \( \mu \)M. There was no detectable stereoselectivity in the transport activities by OATP1B3 and the inhibition potency of rifampicin. The uptake of fexofenadine enantiomers (\( \mu \)L/mg protein/5 min) was similar in mock vector-transfected and OATP1B1-HEK cells, at 1.92±0.19 and 2.17±0.15 for \( R \)-fexofenadine, and 1.78±0.11 and 2.00±0.18 for \( S \)-fexofenadine, respectively (mean±S.E., \( n = 3–6 \)).

**Effect of rifampicin on the uptake of \( R \)- and \( S \)-fexofenadine by OAT3 and MATE1**

The uptake of fexofenadine enantiomers was also determined in OAT3- and MATE1-HEK in the presence and absence of rifampicin or the representative inhibitors (benzylpenicillin for OAT3 and tetraethylammonium for MATE1 and MATE2-K) (Figure 4). Compared with mock vector-transfected cells, OAT3- and MATE1-HEK cells showed significantly greater uptake of fexofenadine enantiomers, which was markedly inhibited by the representative inhibitors, but not by rifampicin at 80 \( \mu \)M.

**Effect of rifampicin on the basal-to-apical transport of \( R \)- and \( S \)-fexofenadine in Caco-2 cells**

The basal-to-apical transport of fexofenadine across a monolayer of Caco-2 cells was 7.1-fold higher than the apical-to-basal transport (\( P_{\text{app}} 4.97±0.16 \times 10^6 \text{versus} 0.70±0.01 \times 10^6 \text{cm/s} \)). Verapamil significantly inhibited this basal-to-apical transport by 61%, but did not affect the apical-to-basal transport (data not shown). Celiprolol was used as the reference P-gp substrate (Figure 5A). Rifampicin significantly inhibited the transport of \( R \)- and \( S \)-fexofenadine with similar potency (Figure 5B). It also inhibits the basal-to-apical transport of celiprolol, but the inhibition potency for
celiprolol was lower than that for fexofenadine enantiomers (Figure 5). There was no detectable stereoselectivity in the basal-to-apical transport of \( R\)- and \( S\)-fexofenadine (Figure 5).

**Stereoselectivity of \( R\)- and \( S\)-fexofenadine binding to human H1 receptor**

Transient transfection of human H1 receptor cDNA induces specific binding of \(^3\text{H}\)-mepyramine with a \( K_d \) and \( B_{\text{max}} \) of 3.7±0.5 nM and 3.8±0.1 pmol/mg protein, respectively. The competition of fexofenadine with \(^3\text{H}\)-mepyramine binding was examined, through which the \( K_i \) values of \( R\)- and \( S\)-fexofenadine were determined to be 30±3 and 13±3 nM, respectively (Figure 6A). Kinetic analysis of the inhibition \(^3\text{H}\)-mepyramine binding by fexofenadine enantiomers were also performed (Figure 6B). Based on the dissociation of \(^3\text{H}\)-mepyramine (\( k_{\text{eff}} = 1.43 \text{ min}^{-1} \)), the association constant (\( k_{\text{on}} \)) and dissociation constant (\( k_{\text{off}} \)) of \( R\)- and \( S\)-fexofenadine were calculated to be 0.86±0.13 and 0.92±0.02 mL/nmol/min, and 0.032±0.008 and 0.017±0.002 min\(^{-1}\), respectively.
Discussion

The present study examined the effect of single and multiple doses of rifampicin on the pharmacokinetics of fexofenadine enantiomers in healthy subjects to demonstrate the impact of OATP1B3 inhibition.

A single dose of rifampicin significantly decreased the CL_{tot}/F and CL_{r} of fexofenadine enantiomers. Based on the reported contribution of urinary excretion to systemic elimination [38%, (Lappin et al., 2010)], the magnitude of the interaction of rifampicin with the CL_{tot}/F of fexofenadine cannot be explained only by the reduction in CL_{r}. Coadministration of rifampicin also inhibits the hepatic elimination of fexofenadine and/or increases its bioavailability. Because of a negligible hepatic first-pass effect, the bioavailability of fexofenadine, which was reported to be 0.30 at its therapeutic dose (Lappin et al., 2010), is attributable to its absorption and solubility in the intestine. Given that rifampicin increases the bioavailability of fexofenadine, the inhibition of intestinal efflux can be the underlying mechanism.

To evaluate the relevance of the inhibition of hepatic elimination by rifampicin, \textit{in vitro} inhibition studies were conducted in cryopreserved human hepatocytes and cDNA transfected cells. Two batches of cryopreserved hepatocytes were used in this study. The two batches of hepatocytes showed very similar transport activity of the OATP1B1 reference substrate, but hepatocytes from batch number 03-013 showed 5-fold lower transport activity of the OATP1B3 reference substrate than batch TDH (Figure 3A). The uptake of fexofenadine by TDH hepatocytes but not by 03-013 hepatocytes was inhibited by rifampicin (Figure 3A), suggesting that rifampicin-sensitive uptake of fexofenadine in the hepatocytes could be related to their OATP1B3 activity. We speculate that OATP1B3 makes a greater contribution than OATP1B1 to rifampicin-sensitive uptake in hepatocytes. Although the specific uptake of fexofenadine was not observed in OATP1B1- and OATP2B1 expressing HEK cells, this does not exclude their involvement in the hepatic uptake of fexofenadine enantiomers. In fact, a transcellular transport study using OATP1B1 and MRP2 double transfectants clearly showed that transfection of OATP1B1 is required to mediate directional transport of fexofenadine in MDCK cells (Matsushima et al., 2008a). Specific uptake of fexofenadine by
OATP2B1 was reported previously in OATP2B1-expressing *Xenopus laevis* oocytes, and HEK293 cells (Nozawa et al., 2004; Imanaga et al., 2011). As rifampicin was given orally, the *in vivo* relevance of the interactions in hepatic elimination should be evaluated using the unbound rifampicin concentration at the inlet to the liver, which was estimated to be a maximum of 4.6 μM at the dose of 600 mg (Maeda et al., 2011). Given the *in vitro* inhibition potency of rifampicin (Figure 3B), this concentration is sufficient to cause a marked inhibition of OATP1B3 *in vivo*. Assuming a negligible effect of rifampicin on intestinal absorption, the hepatic clearance of fexofenadine enantiomers is decreased to 4% of the control values by coadministration of rifampicin.

Although the CLr of fexofenadine was significantly decreased by rifampicin (Table 1), this effect is not explained by an inhibition of the candidate transporters OAT3 and MATE1 (Tahara et al., 2006; Matsushima et al., 2009), as rifampicin did not show any inhibitory effect on these transporters (Figure 4). The absence of an effect of rifampicin on OAT3 is consistent with clinical data showing that a simultaneous dose of rifampicin did not affect the renal clearance of another OAT3 substrate, pravastatin (Maeda et al., 2011). Tubular secretion of fexofenadine may involve other unknown transporters that are sensitive to rifampicin. Based on the observation that probenecid, a potent inhibitor of OAT3, inhibited the tubular secretion of fexofenadine almost completely (Yasui-Furukori et al., 2005; Tahara et al., 2006), the site of interaction with rifampicin may be the luminal efflux process. Further studies are necessary to elucidate the mechanism of this DDI.

In addition to the hepatic and renal elimination, the effect of rifampicin on the CLtot/F of fexofenadine may involve inhibition of P-gp in the small intestine. We found that rifampicin inhibited the basal-to-apical transport of fexofenadine enantiomers in Caco-2 cells (Figure 5). Tachibana et al. proposed an apparent volume of the small intestine lumen based on published DDI data to associate the DDI risk for P-gp and CYP3A4 (11–28 L and 2.8–9.4 L, respectively) with the doses of inhibitors (Tachibana et al., 2009). When a volume of 11–28 L is used, the estimated concentration of rifampicin in the lumen is 26–66 μM, which is high enough to cause a moderate inhibition of P-gp-mediated efflux of fexofenadine in the small intestine. Notably, the effect of rifampicin on P-gp will be substrate
dependent, as the same concentration did not affect the basal-to-apical transport of celiprolol (Figure 5).

Hamman et al. reported that multiple doses of rifampicin significantly increased the CL_{tot}/F of fexofenadine (Hamman et al., 2001). It is well known that multiple doses of rifampicin induce expression of P-gp in the small intestine (Shimizu et al., 2006) and consequently lower bioavailability of P-gp substrates including fexofenadine (Hamman et al., 2001; Akamine et al., 2012). Rifampicin also induces OATP1B1 expression in primary cultured human hepatocytes (Sahi et al., 2006), and this may contribute to reducing the AUC of fexofenadine. In this study, even after multiple doses, when the two drugs were given simultaneously, rifampicin significantly decreased the CL_{tot}/F of fexofenadine although the magnitude of the interaction on the CL_{tot}/F was slightly blunted compared with that after a single dose (Figure 2). Thus, the impact on inhibition of drug transporters by rifampicin surpasses its impact on induction of P-gp and OATP1B1. Multiple doses of rifampicin decrease its AUC; however, the C_{max} of rifampicin in subjects given multiple doses over 6 days was decreased by at most one-third of the corresponding value on day 1 (Acocella, 1978) and remained high enough to cause a significant inhibition of the hepatic uptake transporter. Thus, during rifampicin therapy, attention must be paid to the intervals of administration of rifampicin and victim drugs, particularly OATP substrates, as these determine the direction of the DDI, i.e., an increase versus a decrease in the systemic exposure of victim drugs.

Consistent with previous reports (Miura et al., 2007; Tateishi et al., 2008; Sakugawa et al., 2009; Akamine et al., 2012), the pharmacokinetics of fexofenadine showed stereoselectivity (Table 1). Notably, the stereoselectivity in CL_{tot}/F persisted after a single dose of rifampicin, but diminished after multiple doses, while the stereoselectivity in CL_{r} persisted after both single and multiple doses (Table 1). We found stereoselectivity for the unbound fractions of fexofenadine enantiomers in the plasma. The fact that the magnitude of the difference in the unbound fraction of fexofenadine enantiomers (1.8) is similar to the difference in CL_{r} (1.7) excludes stereoselectivity in the intrinsic tubular secretion clearance. This is in good agreement with in vitro data (Figure 3). The stereoselectivity of the CL_{tot} and F remains debatable as the CL_{tot} and F could not be separately evaluated in this study.
Stereoselectivity was not observed in the uptake of fexofenadine enantiomers by OATP1B3 (Figure 3). Thus, the in vivo hepatic uptake clearance, which is the product of the unbound fraction in the plasma and intrinsic influx clearance, will show stereoselectivity. Explanation of the stereoselective effect on the CL<sub>tot</sub>/F of multiple doses of rifampicin requires the involvement of an additional stereoselective factor that is induced under treatment with multiple doses of rifampicin. In Caco-2 cells, the basal-to-apical transport of fexofenadine enantiomers was identical, indicating similar activities of P-gp for fexofenadine enantiomers (Figure 5). OATP2B1 is considered to mediate the uptake of fexofenadine from the intestinal lumen to epithelial cells (Imanaga et al., 2011). However, since specific uptake of fexofenadine enantiomers by OATP2B1 could not be detected in our OATP2B1-expressing cells (data not shown), the stereoselectivity of the transport activity by OATP2B1 remains unknown. Separate evaluation of CL<sub>tot</sub> and F is necessary to elucidate the mechanism underlying the stereoselectivity of fexofenadine pharmacokinetics.

The impact of the stereoselectivity on the pharmacological effect of fexofenadine is debated. Taking the unbound fraction into consideration, the AUC of the unbound plasma fexofenadine enantiomers is similar. Although Robbins et al. reported that the two enantiomers are pharmacologically identical (Robbins et al., 1998), in vitro binding assays demonstrated that the inhibition constant of S-fexofenadine against mepyramine binding to H1 receptor is 2-fold lower than that of R-fexofenadine (Figure 6). Therefore, S-fexofenadine shows larger receptor occupancy, and consequently makes a greater contribution to the pharmacological response than R-fexofenadine. Multiple doses of rifampicin diminished the difference in AUC of fexofenadine enantiomers, thereby increasing the contribution of S-fexofenadine to the pharmacological effect.

In conclusion, rifampicin interacts with fexofenadine at multiple sites in the body, with a simultaneous dose inhibiting the hepatic and renal transporters (OATP1B3 and an unknown transporter, respectively), and presumably the intestine efflux transporter P-gp, all of which contribute to increasing the AUC of fexofenadine to surpass the effect of the induction of P-glycoprotein elicited by multiple doses.
Authorship Contributions

Participated in research design: Kusuhara, Yasui-Furukori, Akamine, Uno, and Sugiyama

Conducted experiments: Miura, Yasui-Furukori, Yoshida, Yokochi, Fukizawa, Ikejiri, and Kanamitsu

Performed data analysis: Kusuhara, Miura, Yasui-Furukori, Akamine, Uno, and Sugiyama

Wrote or contributed to the writing of the manuscript: Kusuhara, Uno, and Sugiyama
References


Tahara H, Kusuhara H, Fuse E, 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.


Footnote

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Figure Legends

Figure 1 Effect of single and multiple doses of rifampicin on the plasma concentrations of fexofenadine enantiomers in healthy subjects

Healthy volunteers received fexofenadine alone (circles) or with rifampicin as a single dose (squares) or multiple doses (triangles). Plasma concentrations of $R$- and $S$-fexofenadine (closed and open symbols, respectively) were determined by HPLC. Each point represents the mean ± SE ($n = 12$).

Figure 2 Effect of single and multiple doses of rifampicin on the $CL_{tot}/F$ and $CL_r$ of fexofenadine enantiomers in healthy subjects

$CL_{tot}/F$ and $CL_r$ of fexofenadine enantiomers were calculated as described in Methods. The plots represent the $CL_{tot}/F$ and $CL_r$ of $S$- and $R$-fexofenadine for individual subjects; the points representing the same individual are connected to one another by dotted lines. The horizontal line in each column indicates the mean values. *$P<0.05$, **$P<0.01$, ***$P<0.001$ between control phase and rifampicin phase.

Figure 3 Effect of rifampicin on the uptake of $R$- and $S$-fexofenadine by cryopreserved hepatocytes (A) and by OATP1B3-HEK (B)

(A) Cryopreserved human hepatocytes (lot TDH and lot 03-013) were incubated at 37 °C for 2 min (estrone-3-sulfate 18 nM and CCK-8 10 nM) or 5 min (fexofenadine, 5 μM) in the presence and absence of rifampicin (RIF) at designated concentrations. (B) Mock vector-transfected and OATP1B3-overexpressing HEK293 cells were incubated at 37 °C for 10 min with fexofenadine enantiomers (5 μM) in the presence and absence of rifampicin at designated concentrations. * Significantly different ($P<0.05$) from the uptake in control groups. Each bar represents the mean ± SE ($n = 3$ or $4$). Where bars are not shown, the S.E. is contained within the limits of the symbol.
**Figure 4 Effect of rifampicin on the uptake of R- and S-fexofenadine by OAT3- and MATE1- and MATE2-K HEK**

Mock vector-transfected and OAT3-, MATE1- and MATE2-K-overexpressing HEK293 cells were incubated at 37 °C for 5 min with fexofenadine enantiomers (5 μM) in the presence or absence of rifampicin or representative inhibitors (benzylpenicillin 10 mM for OAT3, tetraethylammonium 10 mM for MATE1 and MATE2-K) at designated concentrations. *, **: P<0.05, 0.01 versus R- or S-fexofenadine uptake by mock vector-transfected cells; † P<0.01 versus R- or S-fexofenadine uptake by cells expressing the corresponding transporter (OAT3 or MATE1) without inhibitors. Each bar represents the mean ± SE (n = 3 or 4).

**Figure 5 Effect of rifampicin on the basal-to-apical transport of celiprolol (A) and R- and S-fexofenadine (B) across a monolayer of Caco-2 cells**

Basal-to-apical transport of celiprolol (2 μM, A) and fexofenadine enantiomers (4.5 μM, B) for 90 min across a monolayer of Caco-2 cells was determined at 37 °C in the presence or absence of rifampicin at designated concentrations. The cyclosporin A concentration in panel A was 20 μM. * Significantly different (P<0.05) from the transport in control groups. Each bar represents the mean ± SE (n = 3).

**Figure 6 Evaluation of R- and S-fexofenadine binding to human H1 receptor**

(A) Competition of R- and S-fexofenadine against [³H]- mepyramine binding to membranes expressing human H1 receptor was determined (B) [³H]- mepyramine association kinetics were determined in the presence or absence of R- and S-fexofenadine (200 and 100 nmol/L, respectively). Solid lines represent the fitted line. Each symbol represents the mean ± SE (n = 3).
Table 1  Pharmacokinetic parameters of the pharmacokinetics of fexofenadine enantiomers in control and rifampicin-treated subjects

Data are shown as mean (95% confidence interval); \( t_{\text{max}} \) data are shown as median (range).

\* \( P<0.05 \), \*\* \( P<0.01 \), \*\*\* \( P<0.001 \) between control phase and rifampicin phase.

\+ \( P<0.05 \), \+\+ \( P<0.01 \), \+\+\+ \( P<0.001 \) between S-fexofenadine and R-fexofenadine.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Single dose</th>
<th>Multiple doses</th>
<th>Ratio to control (Single dose)</th>
<th>Ratio to control (Multiple doses)</th>
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<tr>
<td>( t_{1/2} ) (h)</td>
<td>3.3 (2.8–3.8)†</td>
<td>3.1 (2.8–3.5)†</td>
<td>2.9 (2.5–3.3)</td>
<td>1.02 (0.82–1.21)</td>
<td>0.92 (0.76–1.09)</td>
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<td>( t_{\text{max}} ) (h)</td>
<td>1.8 (1.0–4.0)</td>
<td>1.8 (1.0–3.0)</td>
<td>2.0 (1.0–4.0)</td>
<td>1.41 (0.94–1.88)</td>
<td>1.38 (0.85–1.92)</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (ng/mL)</td>
<td>127 (92–163)†††</td>
<td>443 (371–515)***†††</td>
<td>318 (252–385)***</td>
<td>4.15 (2.97–5.33)</td>
<td>2.94 (2.22–3.66)</td>
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<tr>
<td>( S)-fexofenadine ( AUC_{0-\infty} ) (ng⋅h/mL)</td>
<td>698 (459–937)***†††</td>
<td>2489 (2100–2877)***†††</td>
<td>1674 (1400–1949)***†</td>
<td>4.56 (3.19–5.94)</td>
<td>3.13 (2.13–4.13)</td>
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<tr>
<td>( CL_{\text{pd}}/F ) (L/h)</td>
<td>56 (41–70)†††</td>
<td>13 (10–17)***††</td>
<td>19 (16–22)***</td>
<td>0.30 (0.20–0.40)</td>
<td>0.45 (0.27–0.63)</td>
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<td>( A_{\text{e0–24}} ) (mg)</td>
<td>3.8 (2.8–4.8)††</td>
<td>5.4 (4.3–6.6)*** ††</td>
<td>5.1 (3.8–6.4)*** †</td>
<td>2.18 (0.87–3.50)</td>
<td>1.92 (0.81–3.02)</td>
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<tr>
<td>( CL_{\text{r}} ) (L/h)</td>
<td>6.6 (4.2–8.9)***</td>
<td>2.5 (1.6–3.4)***</td>
<td>3.0 (2.3–3.7)***</td>
<td>0.49 (0.29–0.64)</td>
<td>0.69 (0.38–0.99)</td>
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<tr>
<td>( R)-fexofenadine ( t_{1/2} ) (h)</td>
<td>3.8 (3.3–4.4)</td>
<td>3.5 (3.2–3.9)</td>
<td>3.2 (2.7–3.6)</td>
<td>0.96 (0.84–1.07)</td>
<td>0.86 (0.74–0.98)</td>
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<tr>
<td>( t_{\text{max}} ) (h)</td>
<td>1.8 (0.5–4.0)</td>
<td>2.0 (1.0–4.0)</td>
<td>2.0 (1.0–4.0)</td>
<td>1.85 (0.67–3.04)</td>
<td>1.59 (0.90–2.28)</td>
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<tr>
<td>( C_{\text{max}} ) (ng/mL)</td>
<td>160 (117–202)</td>
<td>502 (427–577)***</td>
<td>331 (261–401)**</td>
<td>3.65 (2.73–4.57)</td>
<td>2.46 (1.74–3.18)</td>
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<td>AUC(_{0–\infty}) (ng·h/mL)</td>
<td>CL(_{tot/F}) (L/h)</td>
<td>CL(_{r}) (L/h)</td>
<td>(R/S) ratio of AUC</td>
<td>(R/S) ratio of CL(_{renal})</td>
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<td>1011 (695–1327)</td>
<td>37 (28–46)</td>
<td>3.9 (2.5–5.4)</td>
<td>1.50 (1.35–1.65)</td>
<td>0.60 (0.55–0.66)</td>
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<td>3243 (2759–3727)**</td>
<td>10 (8–12)**</td>
<td>1.5 (1.1–2.0)**</td>
<td>1.32 (1.23–1.43)</td>
<td>0.63 (0.59–0.66)</td>
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<tr>
<td></td>
<td>1922 (1635–2210)**</td>
<td>16 (14–19)**</td>
<td>1.8 (1.3–2.2)*</td>
<td>1.17 (1.05–1.29)**</td>
<td>0.59 (0.52–0.66)</td>
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<tr>
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<td>3.86 (2.92–4.80)</td>
<td>0.32 (0.23–0.41)</td>
<td>0.50 (0.31–0.70)</td>
<td>0.91 (0.80–1.03)</td>
<td>1.07 (0.95–1.19)</td>
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<tr>
<td></td>
<td>2.40 (1.62–3.17)</td>
<td>0.55 (0.37–0.73)</td>
<td>0.64 (0.41–0.87)</td>
<td>0.80 (0.70–0.91)</td>
<td>1.00 (0.83–1.16)</td>
</tr>
</tbody>
</table>

\(R/S\) ratios indicate relative comparisons between different parameters.

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Figure 1
Figure 2

**S-fexofenadine**

- **CL_{tot}/F (L/hr)**
- **CL_{r} (L/hr)**

**R-fexofenadine**

- **CL_{tot}/F (L/hr)**
- **CL_{r} (L/hr)**
Figure 3

A  Lot TDH

B  Lot03-013
Figure 4

![Graph showing uptake (mL/5 min/mg protein) for mock, OAT3, MATE1, and MATE2-K under different conditions.](image-url)
Figure 5

A

![Bar graph showing Papp (X10^6 cm/s) for rifampicin at different concentrations (20, 100, 250 μM) and control. The graph includes a control group and shows significant differences marked with an asterisk.]

B

![Bar graph showing Papp (X10^6 cm/s) for R-fexofenadine and S-fexofenadine at different concentrations (20, 100, 250 μM) and control. The graph includes a control group and shows significant differences marked with an asterisk.]

Figure 6

A

[\textsuperscript{3}H]mepyramine specific binding (% of control)

concentration (nM)

[R]-fexofenadine
[S]-fexofenadine

B

[\textsuperscript{3}H]mepyramine specific binding (fmol)

time (min)

control
[R]-fexofenadine
[S]-fexofenadine