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PHARMACOKINETICS AND DISPOSITION

Effects of one-time apple juice ingestion on the pharmacokinetics of fexofenadine enantiomers

Yumiko Akamine · Masatomo Miura · Hisakazu Komori · Shun Saito · Hiroyuki Kusuhara · Ikumi Tamai · Ichiro Ieiri · Tsukasa Uno · Norio Yasui-Furukori

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Abstract
Purpose We examined the effect of a single apple juice intake on the pharmacokinetics of fexofenadine enantiomers in healthy Japanese subjects.
Methods In a randomized two phase, open-label crossover study, 14 subjects received 60 mg of racemic fexofenadine simultaneously with water or apple juice. For the uptake studies, oocytes expressing organic anion-transporting polypeptide 2B1 (OATP2B1) were incubated with 100 μM (*R*)- and (*S*)-fexofenadine in the presence or absence of 10 % apple juice.
Results One-time ingestion of apple juice significantly decreased the area under the plasma concentration–time curve (AUC_{0-24}) for (*R*)- and (*S*)-fexofenadine by 49 and 59 %, respectively, and prolonged the time to reach the maximum plasma concentration (t_{max}) of both enantiomers ($P<0.001$). Although apple juice greatly reduced the amount of (*R*)- and (*S*)-fexofenadine excretion into urine (Ae_{0-24}) by 54 and 58 %, respectively, the renal clearances of both enantiomers were unchanged between the control and apple juice phases. For in vitro uptake studies, the uptake of both fexofenadine enantiomers into OATP2B1 complementary RNA (cRNA)-injected oocytes was significantly higher than that into water-injected oocytes, and this effect was greater for (*R*)-fexofenadine. In addition, apple juice significantly decreased the uptake of both enantiomers into OATP2B1 cRNA-injected oocytes.
Conclusions These results suggest that OATP2B1 plays an important role in the stereoselective pharmacokinetics of fexofenadine and that one-time apple juice ingestion probably inhibits intestinal OATP2B1-mediated transport of both enantiomers. In addition, this study demonstrates that the OATP2B1 inhibition effect does not require repeated ingestion or a large volume of apple juice.

Keywords Enantiomer · Fexofenadine · Apple juice · OATP2B1 · Pharmacokinetics

Tsukasa Uno is a former member of the University of the Ryukyus.

Y. Akamine · T. Uno
Department of Hospital Pharmacy, Faculty of Medicine, University of the Ryukyus, Okinawa, Japan

Y. Akamine · I. Ieiri
Department of Clinical Pharmacokinetics, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan

M. Miura (✉)
Department of Pharmacy, Akita University Hospital, 1-1-1 Hondo, Akita 010-8543, Japan
e-mail: m-miura@hos.akita-u.ac.jp

H. Komori · S. Saito · I. Tamai
Faculty of Pharmacy, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa, Japan

H. Kusuhara
Laboratory of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan

N. Yasui-Furukori
Department of Neuropsychiatry, Hirosaki University School of Medicine, Hirosaki, Japan

Introduction

Drug interactions mediated by organic anion-transporting polypeptides (OATPs) have been increasingly recognized as important clinical events that may significantly alter the bioavailability of orally administered drugs and total body clearance [1, 2]. OATPs are membrane influx transporters expressed in major organs related to drug distribution, absorption, and excretion, such as the blood–brain barrier, small intestine, liver, and kidney [2]. Therefore, OATP-mediated drug interactions may occur in various organs. Of the 11 human OATP transporters, OATP1B1 and

OATP1B3, which are expressed on the sinusoidal membrane of hepatocytes, facilitate the uptake of their substrate drugs into the liver. Rifampicin, an inhibitor of three OATPs, increases the plasma concentrations of several OATP substrate drugs including atrasentan, bosentan, glyburide, repaglinide, and fexofenadine [3–8].

Fruit juices are known to cause drug interactions with OATP substrate drugs [9]. For example, both grapefruit and orange juices decrease the oral bioavailability of OATP1A2 and/or OATP2B1 substrate drugs such as aliskiren, celiprolol, talinolol, atenolol, ciprofloxacin, and fexofenadine [10–16]. OATP1A2 and OATP2B1 are expressed on the luminal membrane of small intestinal enterocytes and potentially participate in the active absorption of drugs [2]. Both of these juices have been found to inhibit OATP1A2 and OATP2B1 in vitro [17–19]; therefore, inhibition of OATP-mediated intestinal uptake is currently considered the mechanism underlying a significant reduction in the oral bioavailability of substrate drugs. In addition to grapefruit and orange juices, apple juice also significantly decreases the bioavailability of drugs such as aliskiren and fexofenadine [10, 16]. Although apple juice had no effect on OATP1A2 activity in an in vitro study [16], a recent study showed that apple juice strongly inhibits OATP2B1 activity in vitro [20–22]. Since apple juice is a more potent OATP2B1 inhibitor than grapefruit or orange juice in vitro [22], it is believed that the primary mechanism of interaction with apple juice also involves the inhibition of OATP2B1-influx transport in the small intestine.

The disposition of fexofenadine enantiomers after racemic dosing shows stereoselectivity with the plasma concentration of (*R*)-fexofenadine approximately 1.5-fold higher than that of the (*S*)-enantiomer [23–26]. Fexofenadine pharmacokinetics primarily depend on the activities of multiple transporters including P-glycoprotein (P-gp), OATPs, and multidrug and toxic compound extrusion 1 (MATE1) [27, 28]. While the activity of both enantiomers was identical in P-gp, OATP1B3, OAT3, or MATE1-expressing cells [8], stereoselectivity was observed for the unbound fractions of fexofenadine enantiomers in plasma, which could reasonably explain the difference in renal clearance of fexofenadine enantiomers. Previous in vivo studies demonstrated that itraconazole, verapamil, and rifampicin significantly increased the concentrations of both enantiomers and also affected the stereoselectivity of fexofenadine pharmacokinetics [8, 24, 25]. Therefore, it is possible that other transporters may also contribute to the stereoselective pharmacokinetics of fexofenadine.

Meanwhile, we reported that *SLCO2B1* (encoding OATP2B1) polymorphisms are associated with the pharmacokinetics of fexofenadine enantiomers [29]. However, it remains unclear to what extent OATP2B1-mediated transport contributes to the stereoselectivity of fexofenadine. As the stereoselective disposition of fexofenadine is also promoted by OATP2B1-mediated transport, apple juice may influence the stereoselective pharmacokinetics of fexofenadine enantiomers. In addition, although racemic fexofenadine exhibited reduced bioavailability after repeated high-volume (1,200 mL) ingestion of apple juice [16], there is no information on the effect of one-time ingestion of apple juice.

Therefore, we examined whether one-time apple juice ingestion affects fexofenadine enantiomer pharmacokinetics and whether apple juice alters the stereoselectivity of fexofenadine absorption. Subsequently, we conducted in vitro studies with OATP2B1 complementary RNA (cRNA)-injected oocytes in support of the clinical data.

Methods

Subjects

Most of the 14 healthy Japanese volunteers (10 males and 4 females) used in this study had participated in our previous study [29]. Each subject was deemed physically healthy by a clinical examination and routine laboratory testing and had no history of significant medical illnesses or hypersensitivity to any drugs. The volunteers had a mean (±standard deviation (SD)) age of 24.9 (±5.7) years (range 20–42 years) and a mean body weight of 57.5 (±8.3) kg (range 44–70 kg). This study was approved by the Ethics Committee of the Hirosaki University School of Medicine, and all subjects gave written informed consent before participating.

Study design

A randomized crossover study design in two phases was conducted in intervals of at least 2 weeks. Following an overnight fast, healthy subjects simultaneously received 60 mg of racemic fexofenadine hydrochloride (Allegra®; Sanofi-Aventis K.K., Tokyo, Japan) with 400 mL of water or apple juice at 9:00 A.M. The extract from tablets (Allegra®) did not have optical activity; as a result, fexofenadine was administered as a racemic mixture, i.e., a 50/50 mixture of enantiomers, where the dose was 30 mg for each fexofenadine enantiomer. The apple juice (Shiny Apple®; Aomoriken Ringo Juice Co. Ltd., Aomori, Japan) used in this study was of normal strength. Volunteers did not ingest any medication, fruit juices, or apple, orange, or grapefruit products for at least 7 days before either of the study phases. No meals or beverages were allowed until 4 h after fexofenadine administration. In addition, the use of alcohol, tea, and coffee was forbidden during the test period.

Plasma and urine collection and determination of fexofenadine enantiomer concentrations

Blood samples (10 mL each) were drawn into heparinized tubes before and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after administration of fexofenadine, and the plasma was immediately separated. Immediately prior to fexofenadine administration, urine was collected to provide a blank sample. After fexofenadine administration, urine samples were collected during the following 24-h period. The plasma and urine samples were stored at −20 °C until assayed.

Fexofenadine concentrations in plasma and urine were determined using an HPLC method that had been developed in our laboratory [30]. In brief, following the addition of diphenhydramine (50 ng) in methanol (10 μL) as an internal standard to 400 μL of plasma, the plasma sample was diluted with 600 μL of water and was vortexed for 30 s. For urine samples, diphenhydramine (50 ng) in methanol (10 μL) was added to a 100-μL urine sample, and the sample was then diluted with 900 μL of water. These sample mixtures were applied to an Oasis HLB extraction cartridge that had been previously activated with methanol and water (1.0 mL each). The cartridge was successively washed with 1.0 mL of water and 1.0 mL of 40 % methanol in water followed by elution with 1.0 mL of 100 % methanol. Eluates were evaporated to dryness under vacuum at 40 °C using a rotary evaporator (Iwaki, Tokyo, Japan). The residue was dissolved in 50 μL of methanol and was vortexed for 30 s. Approximately 50 μL of mobile phase was added, and the sample was vortexed for another 30 s. A 50-μL aliquot of the sample was then processed using the HPLC apparatus. The HPLC column was a Chiral CD-Ph (250 mm×4.6 mm i.d.; Shiseido, Tokyo, Japan), and the mobile phase was 0.5 % KH_2PO_4 (pH 3.5)–acetonitrile (65:35, *v*/*v*). The flow rate was 0.5 mL/min at ambient temperature, and sample detection was performed at 220 nm. The lower limit of quantification was 25 ng/mL for both (*R*)- and (*S*)-fexofenadine. The validated concentration range of this assay for either plasma or urine samples was 25–625 ng/mL for both enantiomers. The within- and between-day coefficients of variation were less than 13.6 %, and accuracy was within 8.8 % over the linear range of both analytes. The plasma and urine samples after apple juice treatment did not have any interfering peaks in the fexofenadine assay. The plasma and urine blank samples that were collected before initiating fexofenadine administration presented no detectable fexofenadine peak in the assay.

Uptake experiments in *Xenopus laevis* oocytes

The preparation of oocytes, in vitro synthesis of OATP2B1 cRNA, and uptake experiments were conducted as described previously [31]. In brief, the construct pGEMHE containing OATP2B1 complementary DNA was used to synthesize cRNA. Defolliculated oocytes were injected with 50 nL of the cRNA solution (1 μg/μL) or water and were then incubated for 3 days at 18 °C in modified Barth's saline [MBS; 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, and 10 mM HEPES, pH 7.4] containing 50 μg/mL gentamicin. For uptake studies, oocytes expressing OATP2B1 were incubated with 100 μM (*R*)- and (*S*)-fexofenadine in the presence or absence of 10 % apple juice for 120 min at 25 °C. Uptake was terminated by washing the oocytes three times with ice-cold MBS. The concentrations of fexofenadine in all of the samples were quantified with a liquid chromatography–tandem mass spectrometry (LC-MS/MS) system consisting of an MDS-Sciex API 3200™ triple quadrupole mass spectrometer (AB SCIEX, Foster City, CA) coupled with a LC-20 AD ultrafast LC system (Shimadzu Company, Kyoto, Japan). The ultrafast liquid chromatography gradient elution was performed using a mobile phase consisting of 0.1 % formic acid and acetonitrile at a flow rate of 0.3 mL/min. The gradient profile was 5.0 % acetonitrile from 0 to 1.25 min, 5.0–95 % acetonitrile from 1.25 to 2.25 min, 95 % acetonitrile from 2.25 to 4.35 min, 95–5.0 % acetonitrile from 4.35 to 4.5 min, and 5.0 % acetonitrile from 4.5 to 5.5 min, for a total run time of 5.5 min for each injection. The retention times were 2.9 min. Mercury MS (C_{18}, 10×4.0 mm, Luna 3 mm, Phenomenex, Torrance, CA) was used as the analytical column. In the LC-MS/MS system, the Turbo Ion Spray interface was operated in positive ion mode at 5,500 V and at 700 °C, and the mass transition (Q1/Q3) of *m*/*z* 502.3/466.3 was used. Analyst software version 1.4 (Applied Biosystems) was used for data manipulation. The uptake clearance (μL/120 min/oocyte) was calculated as the cell-to-medium ratio by dividing the uptake amount by the initial cell concentration in the uptake medium. OATP2B1-mediated uptake rates were obtained after subtraction of the uptake by water-injected oocytes from the uptake by OATP2B1 cRNA-injected oocytes.

Pharmacokinetic data analysis

Pharmacokinetic analysis of fexofenadine enantiomers was performed with a standard, non-compartmental method using WinNonlin (Pharsight Co., Mountain View, CA, version 4.0.1). The maximum plasma concentration (C_{max}) and time to reach C_{max} (t_{max}) were determined directly from the observed data. The elimination rate constant (ke) for fexofenadine was obtained by linear regression analysis using at least three sampling points from the terminal log-linear declining phase to the last measurable concentration. The elimination half-life ($t_{1/2}$) was calculated as 0.693 divided by ke. The area under the concentration-time curve (AUC) from time 0 to the last sampling time ($AUC_{0–24}$) was calculated by the trapezoidal rule. The apparent oral clearance (CL/F) was obtained from the equation $CL/F = dose/AUC_{0–24}$, where the

dose was 30 mg for each fexofenadine enantiomer. Renal clearance (CL_{renal}) was obtained from the following equation: $CL_{renal}=Ae_{0-24}/AUC_{0-24}$, where Ae is the amount of fexofenadine excreted into the urine within a 24-h period.

Statistical analysis

The bioequivalence approach, as recommended by the US Food and Drug Administration [32] for ratios between the control phase and apple juice treatment phases, served to illustrate the intraindividual variability of the pharmacokinetic parameters in 14 subjects. The 90 % confidence interval (CI) for the ratio would fall entirely within the interval 0.8–1.25 to demonstrate a lack of significant effect on the pharmacokinetic parameters chosen. To demonstrate a difference in AUC of 50 % between the two phases with an α error of 5 % and a statistical power of 80 %, the sample size was analyzed assuming a 20 % SD of difference based on the pharmacokinetics of our previous study [26].

The results are expressed as the mean and 95 % CI in Table 1. In the human study (Fig. 1), data are reported as the mean+SD to indicate variability in the samples. In the in vitro study (Fig. 3), data are reported as the mean+standard error of the mean (SEM) to quantify the precision of the mean measurements.

Differences between (*R*)- and (*S*)-fexofenadine pharmacokinetic parameters during the control phase and apple juice phase, in the *S/R* ratio of the AUC_{0-24} during the control phase and apple juice phase, and in the mean difference (%) in the within-subject ratio (e.g., apple juice/control) were analyzed using the paired *t* test. The comparison of t_{max} was performed using the Wilcoxon signed sample test. A *P* value of 0.05 or less was regarded as significant. Geometric mean ratios to corresponding values in the control phase with 95 % CI were used for detection of significant difference. When the 95 % CI did not cross 1.0, the result was also regarded as significant. All data were analyzed with the statistical program SPSS for Windows, version 11.5 J (SPSS Inc. Chicago, III). For uptake studies, data are given as the mean of values obtained in at least three experiments with the standard error. Statistical analyses were performed with the unpaired Student's *t* test, and a probability of less than 0.05 ($P<0.05$) was considered to represent a statistically significant difference.

Results

Plasma pharmacokinetics of fexofenadine enantiomers

None of the enrolled subjects reported any adverse events during the study, and the subjects completed all phases according to the study protocol. The mean (+SD) plasma concentration–time profiles of the fexofenadine enantiomers after a single oral dose of 60 mg of fexofenadine hydrochloride are shown in Fig. 1 for both the control (water) and apple juice-treated phases, and the pharmacokinetic parameters are summarized in Table 1.

Table 1 Effect of apple juice on pharmacokinetic parameters of fexofenadine enantiomers

	Parameters	Control	With apple juice	Ratio to control
(*R*)-fexofenadine	$t_{1/2\ (h)}$	3.8 (3.3, 4.3)††	3.8 (3.4, 4.3)††	1.07 (0.90, 1.25)
	$t_{max\ (h)\ (range)}$	1.5 (0.5–3.0)	2.9 (1.5–4.0)***	2.39 (1.62–3.15)
	$C_{max\ (ng/mL)}$	131 (110, 152)†††	62 (52, 72)***†††	0.52 (0.40, 0.63)
	$AUC_{0-24\ (ng\cdot h/mL)}$	774 (650, 898)†††	364 (305, 423)***†††	0.51 (0.40, 0.62)
	CL/F $_{(L/h)}$	41 (36, 48)†††	95 (70, 120)***†††	2.48 (1.67, 3.30)
	$Ae_{0-24\ (mg)}$	3.6 (2.9, 4.3)	1.5 (1.2, 1.9)***	0.46 (0.33, 0.60)
	$CL_{renal\ (L/h)}$	5.1 (3.9, 6.2)†††	4.4 (3.4, 5.4)†††	1.14 (0.61, 1.67)
(*S*)-fexofenadine	$t_{1/2\ (h)}$	3.0 (2.5, 3.4)	2.7 (2.2, 3.1)	0.96 (0.75, 1.12)
	$t_{max\ (h)\ (range)}$	1.6 (0.5-4.0)	2.8 (1.5–4.0)***	2.33 (1.60–3.07)
	$C_{max\ (ng/mL)}$	110 (94, 127)	41 (33, 50)***	0.40 (0.31, 0.49)
	$AUC_{0-24\ (ng\cdot h/mL)}$	530 (416, 643)	185 (148, 222) ***	0.41 (0.30, 0.50)
	CL/F $_{(L/h)}$	64 (53, 76)	205 (130, 281) ***	2.95 (1.95, 3.95)
	$Ae_{0-24\ (mg)}$	4.0 (3.3, 4.8)	1.6 (1.2, 2.0)***	0.42 (0.31, 0.53)
	$CL_{renal\ (L/h)}$	8.5 (6.6, 10.4)	9.7 (7.3, 12.1)	1.08 (0.53, 1.63)
S/R ratio of AUC_{0-24}		0.67 (0.62, 0.73)	0.50 (0.45, 0.54)**	0.76 (0.66, 0.86)
S/R ratio of CL_{renal}		1.71 (1.58, 1.84)	2.19 (1.88, 2.50)	1.33 (1.05, 1.61)

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, between control phase and apple juice phase

$^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$, $^{\dagger\dagger\dagger}P<0.001$, between (*R*)- and (*S*)-fexofenadine

Data are shown as mean and 95 % confidence interval; t_{max} data are shown as a median with a range

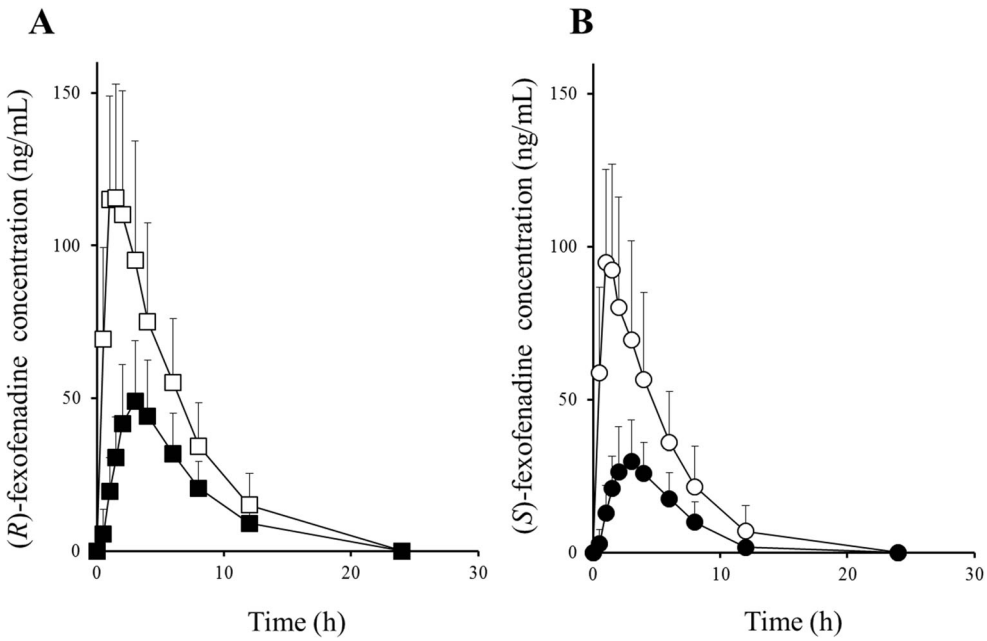

Fig. 1 **a** Mean (+SD) plasma concentration–time curves of (*R*)-fexofenadine following a single oral administration of 60 mg of fexofenadine hydrochloride in 14 healthy volunteers treated with water (*open squares*) or apple juice (*closed squares*). **b** Mean (+SD) plasma concentration–time curves of (*S*)-fexofenadine following a single oral administration of 60 mg of fexofenadine hydrochloride in 14 healthy volunteers treated with water (*open circles*) or apple juice (*closed circles*)

One-time ingestion of apple juice greatly reduced the plasma concentrations of both fexofenadine enantiomers compared with those from the water phase (Fig. 1) and altered all of the pharmacokinetic parameters except $t_{1/2}$ (Table 1). On the other hand, in the water phase, the plasma concentration of (*R*)-fexofenadine at all time points was higher than that of the corresponding (*S*)-enantiomer, and the mean $AUC_{0–24}$ *S/R* ratio was 0.67 (95 % CI, 0.62, 0.73) (Fig. 1 and Table 1).

Apple juice significantly decreased the $AUC_{0–24}$ values of both enantiomers in all of the subjects ($P < 0.001$ for

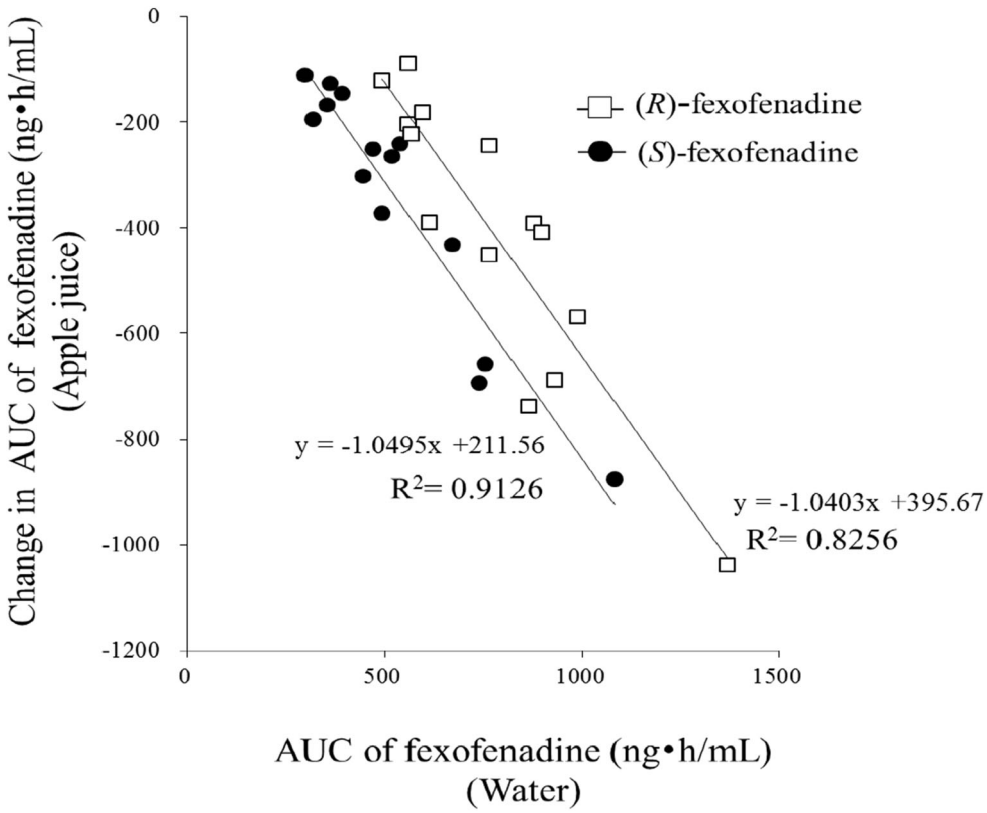

Fig. 2 Change in (*R*)- and (*S*)-fexofenadine $AUC_{0–24}$ by apple juice plotted against $AUC_{0–24}$ during the water phase for each individual ($n = 14$). (*R*)-fexofenadine (*open squares*) and (*S*)-fexofenadine (*closed circles*)

both enantiomers). Additionally, these effects were dependent on the baseline value of each enantiomer, and a highly significant correlation was observed (R^2=0.8256, P<0.001 for (*R*)-fexofenadine; R^2=0.9126, P<0.001 for (*S*)-fexofenadine) (Fig. 2).

Moreover, although there was no significant difference in the mean $t_{1/2}$ of the enantiomers in the control and apple juice-treated phases, the mean t_{max} of both enantiomers was noticeably prolonged by apple juice (P< 0.001 for both enantiomers) (Table 1).

Urinary excretion of fexofenadine enantiomers

In the control phase, the $Ae_{0–24}$ of (*S*)-fexofenadine was slightly higher than that of (*R*)-fexofenadine; however, the difference did not reach statistical significance (P= 0.541) (Table 1). The mean CL_{renal} of (*S*)-fexofenadine was significantly higher than that of (*R*)-fexofenadine (P< 0.001) (Table 1).

Apple juice greatly decreased the mean $Ae_{0–24}$ values of both enantiomers (P< 0.01 for both enantiomers); however, the mean $Ae_{0–24}$ values were not significantly different between the (*R*)- and (*S*)-enantiomers (P= 0.172). In addition, compared to the control, apple juice did not change the mean CL_{renal} of either enantiomer. Apple juice slightly increased the mean S/R ratio of the CL_{renal} from 1.71 (95 % CI, 1.58, 1.84) to 2.19 (95 % CI, 1.88, 2.50), but this difference was not statistically significant (P= 0.081) (Table 1).

In vitro drug transport studies using *Xenopus* oocytes expressing OATP2B1

To determine whether fexofenadine enantiomers were transported by OATP2B1, the uptake of each enantiomer was measured using *Xenopus* oocytes expressing OATP2B1 (Fig. 3). The uptake of (*R*)- and (*S*)-fexofenadine (100 μM) into OATP2B1 cRNA-injected oocytes was significantly higher than the uptake into water-injected oocytes, and the uptake was 2.2 times greater for (*R*)-fexofenadine (P< 0.001) (Fig. 3). In addition, OATP2B1-mediated uptake of each enantiomer was significantly decreased in the presence of 10 % apple juice (P< 0.001 for both enantiomers) (Fig. 3).

Inhibitory effects of apple juice on (*R*)- and (*S*)-fexofenadine

In the human study, apple juice significantly decreased the $AUC_{0–24}$ for (*R*)- and (*S*)-fexofenadine by 49 and 59 %, respectively (P< 0.001 for both enantiomers), and this inhibition effect exhibited significant differences between (*R*)- and (*S*)-fexofenadine (P<0.001) (Table 1). Therefore, apple juice decreased the mean *S/R* ratio of the $AUC_{0–24}$ from 0.67 to 0.50 (95 % CI, 0.45, 0.54) (P<0.01) (Table 1). Meanwhile, in the in vitro study, apple juice significantly decreased OATP2B1-mediated uptake of (*R*)- and (*S*)-fexofenadine by 65 and 64 %, respectively (P< 0.001 for both enantiomers), but the inhibition effect was identical for both enantiomers (P= 0.888) (Fig 3).

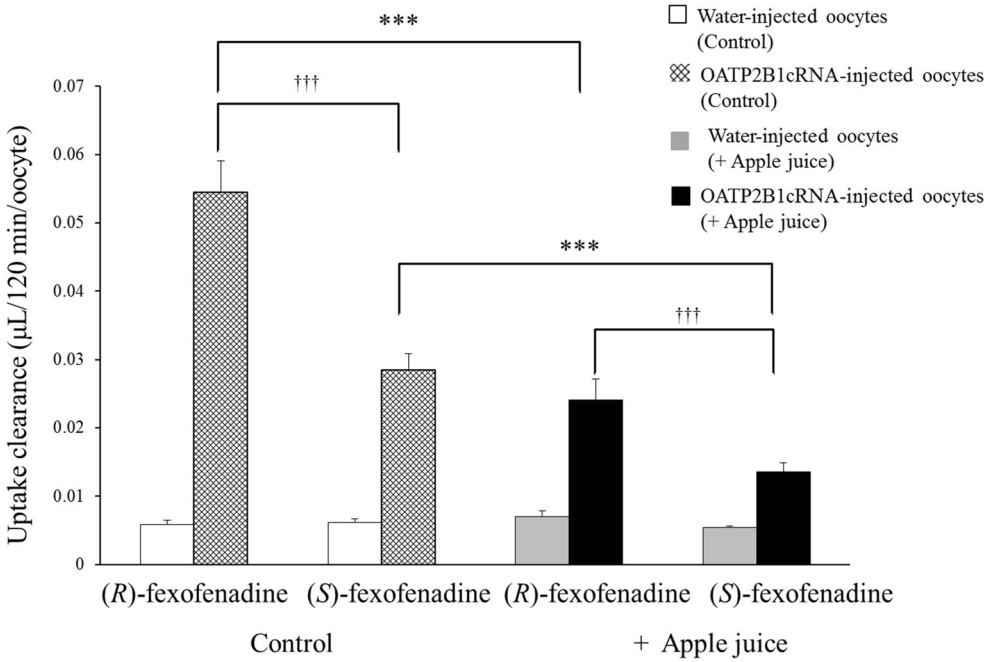

Fig. 3 Uptake clearance of (*R*)- and (*S*)-fexofenadine into *Xenopus* oocytes expressing OATP2B1. Uptake clearance of (*R*)- and (*S*)-fexofenadine (100 μM) into water-injected (*open bars* without apple juice, *gray bars* with apple juice) or OATP2B1 cRNA-injected oocytes (*hatched bars* without apple juice, *closed bars* with apple juice) measured in the presence or absence of 10 % apple juice for 120 min at 25 °C and pH 6.5. Data are shown as the mean+SEM (n=4–9). ***P<0.001, between control phase and apple juice phase. †††P<0.001, between (*R*)-fexofenadine and (*S*)-fexofenadine

Discussion

In this study, the effects of one-time apple juice ingestion on the pharmacokinetics of fexofenadine enantiomers were investigated. This study showed that apple juice significantly decreased the mean C_{max} and $AUC_{0–24}$ of both enantiomers even after a single ingestion (Fig. 1 and Table 1). As this effect of apple juice is attributable to an inhibition of hepatic OATP-influx transport, the plasma concentrations of both enantiomers would have been somewhat enhanced as described previously [3–7]. Therefore, apple juice should inhibit intestinal absorption via OATPs. This supposition is supported by the fact that the mean t_{max} of both enantiomers was significantly prolonged by apple juice (Table 1). The inhibition of influx transporters in the intestine can be a cause of slow absorption [20]. Therefore, it is possible that inhibition of intestinal OATP-influx transport by apple juice leads to a decrease in the distribution volume of both enantiomers and a simultaneous delay in their absorption.

A previous study found that the uptake of fexofenadine was significantly increased in OATP2B1 cRNA-injected oocytes and that this uptake decreased in the presence of apple juice [20]. Similarly [20], in the present study, the uptake of both fexofenadine enantiomers into OATP2B1 cRNA-injected oocytes was significantly higher than into water-injected oocytes (Fig. 3), and apple juice significantly decreased the uptake of both enantiomers into OATP2B1 cRNA-injected oocytes (Fig. 3). Therefore, these results suggest that OATP2B1 plays an important role in the pharmacokinetics of fexofenadine enantiomers and that apple juice may have inhibited OATP2B1-mediated intestinal transport. Furthermore, in the present study, although we did not examine which apple juice ingredients were responsible for the observed effects, our previous in vitro study showed that significant inhibition of OATP2B1 was observed with a mixture of phloridzin, phloretin, hesperidin, and quercetin at concentrations present in apple juice [21]. Thus, further studies may be required to confirm the extent that these ingredients contribute to fexofenadine enantiomer pharmacokinetics.

Additionally, a previous clinical study showed that repeated ingestion of apple juice reduced the AUC of racemic fexofenadine by approximately 70 % of control [16], while our single-intake study showed that the $AUC_{0–24}$ of (*R*)- and (*S*)-fexofenadine decreased by 49 and 59 %, respectively (Table 1). These findings imply that the inhibitory effect of apple juice may increase with repeated high-volume ingestion. However, the present study suggests that one-time ingestion of apple juice might be sufficient for the intestinal OATP2B1 inhibition effect and could possibly be of moderate clinical significance for patients receiving OATP2B1 substrate drugs such as fexofenadine.

On the other hand, apple juice contains many calories. The caloric load of a meal can strongly influence intestinal motility (e.g., gastric emptying, ileocecal transfer) and intestinal water absorption and secretion. However, coadministration of 120 mg of fexofenadine with a high-fat meal had no clinically significant effect on the rate or extent of fexofenadine absorption [33]. Although the t_{max} of fexofenadine was not affected by a high-fat meal [33], we observed that the mean t_{max} of both enantiomers was noticeably prolonged by apple juice. Since t_{max} does not change depending on caloric intake, it is possible that inhibition of intestinal OATP2B1 is the main contributing factor for prolongation of t_{max}.

Moreover, the inhibition effect of apple juice on fexofenadine enantiomer was dependent on the control $AUC_{0–24}$ value of each enantiomer, since there was a high correlation for apple juice causing a greater reduction in subjects with a higher control $AUC_{0–24}$ value (Fig. 2). This result is consistent with a previous report [16] and implies that the higher baseline $AUC_{0–24}$ values observed in the control phase may be due to higher intestinal OATP2B1 activity. A previous in vivo study indicated that a *SLCO2B1* polymorphism contributed to individual pharmacokinetics of fexofenadine since its bioavailability and apple juice effects were significantly higher in *SLCO2B1*1/*1* (1457CC) subjects than those in subjects carrying the **3* (c. 1457C>T) allele [20]. Therefore, it is possible that the individual pharmacokinetics of fexofenadine enantiomers may be affected by OATP2B1 activity and that different reductions of OATP2B1 activity by apple juice may be related to individual apple juice effects on fexofenadine stereoselective pharmacokinetics.

Consistent with previous clinical reports [8, 23–26], the present study demonstrated that the plasma concentration of (*R*)-fexofenadine was higher than the corresponding (*S*)-enantiomer during the control phase (Fig. 1). Interestingly, uptake into OATP2B1 cRNA-injected oocytes was 2.2 times higher for (*R*)-fexofenadine (Fig. 3). In addition, in our previous in vitro study, stereoselectivity in the transport of fexofenadine enantiomers by P-gp, OATP1B3, OAT3, and MATE1-expressing cells was not observed [8]. This result indicates that OATP2B1-mediated transport is stereoselective and that the higher absorption of (*R*)-fexofenadine may be due to higher affinity of OATP2B1 for the (*R*)-enantiomer. These results suggest that OATP2B1 is a key determinant in the stereoselective pharmacokinetics of fexofenadine.

Furthermore, it remains unknown which transporters play a major role in the renal excretion of both enantiomers, but stereoselectivity in renal excretion of fexofenadine was also noted as the CL_{renal} of (*S*)-fexofenadine was 1.7-fold greater for (*R*)-fexofenadine in the control phase. Differences in renal clearance may also be a rationale for the lower AUC of (*S*)-fexofenadine. In addition, the mean $t_{1/2}$ was significantly shorter for (*S*)-fexofenadine compared with the (*R*)-

enantiomer. However, apple juice did not change the CL_{renal} and $t_{1/2}$ of either enantiomer. These results imply that the OATP2B1 inhibition effect by apple juice does not contribute to the stereoselectivity in renal excretion of fexofenadine. Therefore, other transporters and/or factors may be involved in the stereoselective urinary pharmacokinetics. Our previous in vitro study showed that the plasma-unbound fraction of (*S*)-fexofenadine was 1.8-fold higher than that of the (*R*)-enantiomer [8], which is similar to the difference in CL_{renal}. Thus, the plasma-unbound fraction might contribute to the fexofenadine stereoselective pharmacokinetics [8]. Taken together, these findings suggest that the stereoselectivity of fexofenadine pharmacokinetics may occur through a combination of OATP2B1 activity and the plasma-unbound fraction for each enantiomer, resulting in a higher plasma concentration of the (*R*)-fexofenadine enantiomer. However, it is difficult to clarify experimentally whether the stereoselectivity of fexofenadine occurs in the absorption and/or excretion process, since an intravenous fexofenadine formulation has not been produced to date.

In addition, Glaeser et al. [34] reported that OATP1A2 is a critical determinant in the uptake of fexofenadine. Recently, an in vitro study demonstrated that verapamil inhibited the uptake of fexofenadine through OATP1A2-mediated transport [35]. Our previous clinical report showed that verapamil altered the stereoselectivity of fexofenadine [25]. These findings might indicate that OATP1A2 takes part in the stereoselective pharmacokinetics of fexofenadine. However, since there are no in vitro data on the contribution of OATP1A2 to the stereoselective pharmacokinetics of fexofenadine and verapamil is known to be a potent P-gp inhibitor [36], further studies using other OATP1A2 inducers/inhibitors will be needed to confirm the extent of its contributions.

In conclusion, these results suggest that OATP2B1 plays an important role in the stereoselective pharmacokinetics of fexofenadine and that one-time apple juice ingestion probably inhibits intestinal OATP2B1-mediated transport of both enantiomers. In addition, this study demonstrates that OATP2B1 inhibition effects do not require repeated ingestion or a large volume of apple juice.

Conflict of interest The authors have no conflicts of interest in relation to this paper.

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