

Various Drug-Transporters Related to the Fexofenadine Enantiomers Pharmacokinetics

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Doctoral Dissertation

Various Drug-Transporters Related to the
Fexofenadine Enantiomers Pharmacokinetics

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Abbreviations

ABC	ATP-binding cassette
A _e	amount excreted into the urine
AUC	area under the plasma concentration-time curve
BCRP	breast cancer resistance protein
CI	confidence interval
CL/F	oral clearance
CL _{renal}	renal clearance
C _{max}	maximum plasma concentration
cRNA	complementary RNA
CYP	cytochrome P450
HPLC	high pressure liquid chromatography
IQR	interquartile range
k _e	elimination rate constant
LC-MS/MS	liquid chromatography-tandem mass spectrometry
MATE	multidrug and toxic compound extrusion
MRP	multidrug resistance protein
N.S.	not significant
OAT	organic anion transporter
OATP	organic anion transporting polypeptide
OCT	organic cation transporter
PCR-RFLP	polymerase chain reaction-restriction fragment length polymorphism
P-gp	P-glycoprotein

PPI	proton pump inhibitor
SD	standard deviation
SEM	standard error of the mean
SLC	solute carrier
SNP	single nucleotide polymorphism
$t_{1/2}$	elimination half-life
t_{\max}	time to reach maximum plasma concentration
UV	ultraviolet

General Introduction

Drug-transporters play an important role in the clinical pharmacokinetics of many therapeutic agents [1-3]. In particular, ATP-binding cassette (ABC) and the solute carrier (SLC) superfamilies can be major determinants of the pharmacokinetic, safety and efficacy profiles of drugs [1]. They are located in various tissues (Fig. 1) [4], such as the luminal membrane of small intestinal enterocytes, the sinusoidal and canalicular membranes of hepatocytes, and the brush border membranes of proximal tubules, and play an important role in the absorption, distribution and excretion of therapeutic agents, which consequently alter their clinical effects [1-3].

P-glycoprotein (P-gp; also known as MDR1 and ABCB1) belongs to the ABC superfamily and is an efflux pump capable of transporting a wide range of compounds, such as digoxin, paclitaxel, cyclosporine, and HIV protease inhibitors [1,5-7]. P-gp is particularly expressed in the blood-brain barrier, small intestine, liver and kidney, wherein it is related to drug disposition and regulates the absorption and elimination of substrate drugs [1,8,9]. There has been considerable interest in the *ABCB1* gene variation as a predictor of pharmacokinetic and/or treatment outcome of several drugs [10,11]. Moreover, P-gp-mediated transport activity is modulated by inhibition and induction, which can affect the pharmacokinetics [1,8].

In addition, drug interactions mediated by organic anion-transporting polypeptides (OATPs) are also increasingly recognized as important clinical events that may significantly change the bioavailability of orally administered drugs, and total body clearance [1,2]. OATPs belong to the SLC superfamily and are membrane

influx transporters expressed in the major organs related to the drug distribution, absorption and excretion, such as the blood-brain barrier, small intestine, liver, and kidney [2]. Therefore, OATPs mediated-drug interactions may occur at various organs. Of the 11 human OATP transporters, OATP1B1, OATP1B3 and OATP2B1 are expressed on the sinusoidal membrane of hepatocytes and can facilitate the liver uptake of their substrate drugs [2,12-16]. While, OATP2B1 and OATP1A2 are expressed on the luminal membrane of small intestinal enterocytes, potentially participating in the active absorption of drugs [2,17-23].

Meanwhile, it is estimated that about half of all therapeutic agents are chiral, most of these drugs are administered as racemic mixtures, i.e. a 50/50 mixture of its enantiomers [24]. However, the racemate is not simply a mixture of the two enantiomers but rather is a distinct molecular entity with properties quite distinct from those of the two optical isomers [25-29]. Two enantiomers may show enantioselective pharmacokinetic and/or pharmacodynamic profiles [30,31]. There are many studies relative to the stereoselective pharmacokinetics of racemic mixture and most of them are related to cytochrome P450s (CYPs) [24,32]. For example, with the proton pump inhibitors (PPIs) omeprazole and lansoprazole, the plasma concentrations of (*S*)-omeprazole and (*R*)-lansoprazole are higher and are less influenced by CYP2C19 genetic polymorphisms as compared to their corresponding enantiomers [32-37]. This finding has led to the development of esomeprazole and dexlansoprazole, the (*S*)-enantiomer of omeprazole and (*R*)-enantiomer of lansoprazole, respectively, as single enantiomer PPIs. In addition, although many studies on the stereoselective pharmacokinetics related to CYPs have been reported, there has been little information of the stereoselective pharmacokinetics related to drug-transporters.

Fexofenadine, (±)-2-[4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)piperidino]butyl] phenyl]-2-methylpropanoic acid, is one of the most widely used drugs for seasonal allergic rhinitis and chronic urticaria [38]. Metabolism accounts for less than 1% of fexofenadine elimination processes in humans since more than 95% of the drug is excreted in both urine and feces in non-metabolized form [39,40]. Based on several *in vitro* studies, many clinical studies on fexofenadine pharmacokinetics have been performed [40-51]. For example, carbamazepine [41], rifampicin [42,43], St. John's wort [44], itraconazole [45-47], verapamil [48-50] and several fruit juices [51] affected fexofenadine pharmacokinetics. Since fexofenadine is poorly metabolized by CYPs, it has been suggested that drug-transporters play an important role in fexofenadine pharmacokinetics; including P-gp, OATPs, multidrug resistance protein 2 (MRP2), breast cancer resistance protein (BCRP) and multidrug and toxic compound extrusions (MATEs) [40,52-54].

Moreover, fexofenadine is therapeutically administered as a racemic mixture of (*R*)- and (*S*)-enantiomers [55]. Our previous clinical studies have demonstrated that the disposition of fexofenadine enantiomers shows the stereoselectivity; the plasma concentration of (*R*)-fexofenadine in humans is about 1.5-fold higher than that of the corresponding (*S*)-enantiomer [56,57]. Such differences among the pharmacokinetics of fexofenadine enantiomers are likely to be influenced by the difference of the affinity for drug-transporters. In a recent *in vitro* study, cetirizine enantiomers that were applied to Caco-2 cells were transported differently in comparison to one another, and the absorptive permeabilities of (*R*)- and (*S*)-enantiomers were changed in the presence of P-gp inhibitors, such as verapamil and quinidine [58]. In addition to this result, our *in vivo* studies have reported that

P-gp inhibitors, such as itraconazole and verapamil, significantly increase the plasma concentrations of both enantiomers, and the effect on the P-gp-mediated transport of (*S*)-fexofenadine may be greater in comparison to that of (*R*)-enantiomer [59,60]. However, almost clinical studies have been conducted using a racemic mixture of fexofenadine, and consequently, information on the pharmacokinetic parameters of individual fexofenadine enantiomers is lacking. Although P-gp and OATPs may have major influences on fexofenadine pharmacokinetics, whether these transporters have important roles in the stereoselective pharmacokinetics of fexofenadine has not yet been determined. Therefore, we need to confirm the extent of drug-transporters contributing to each fexofenadine enantiomer of pharmacokinetics. In the first chapter, we examined whether drug-transporter polymorphisms influence on the pharmacokinetics of fexofenadine enantiomers. Subsequently, in the second and third chapters, we have evaluated the roles of P-gp and OATPs on the fexofenadine enantiomers pharmacokinetics using these transporter inducers/inhibitors. The present results will lead to further research concerning the chirality of racemic mixtures.

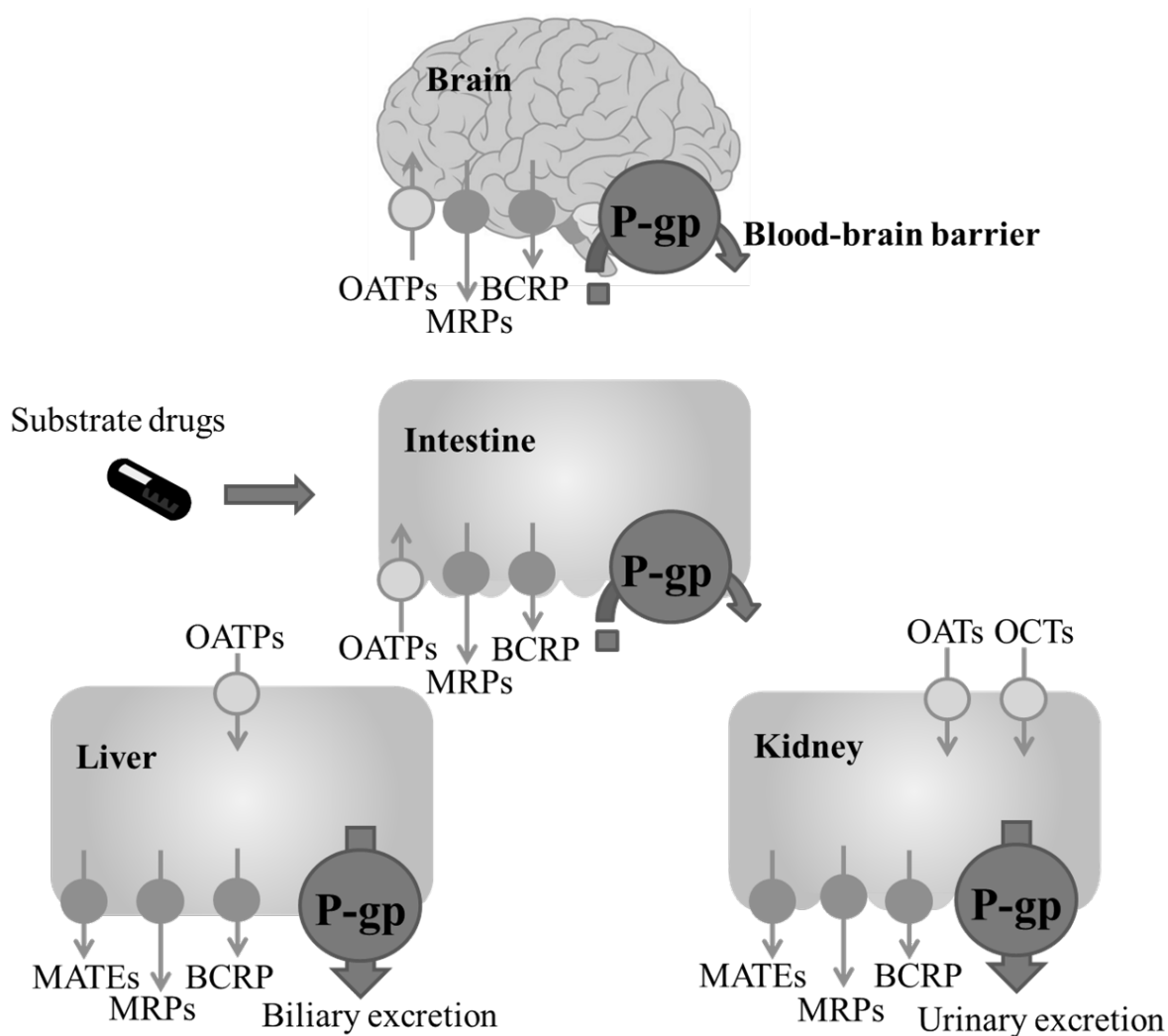


Fig. 1. The roles of drug transporters.

BCRP = breast cancer resistance protein; MATEs = multidrug and toxic compound extrusions; MRPs = multidrug resistance-associated proteins; OATs = organic anion transporters; OATPs = organic anion-transporting polypeptides; OCTs = organic cation transporters; P-gp = P-glycoprotein.

Publications (Including preparations)

This doctoral dissertation is based on following papers.

1. Influence of drug-transporter polymorphisms on the pharmacokinetics of fexofenadine enantiomers.

Akamine Y, Miura M, Sunagawa S, Kagaya H, Yasui-Furukori N, Uno T.
Xenobiotica 2010; 40, 782-789.

(Chapter 1)

2. Carbamazepine differentially affects the pharmacokinetics of fexofenadine enantiomers.

Akamine Y, Miura M, Yasui-Furukori N, Kojima M, Uno T.
Br J Clin Pharmacol 2012; 3, 478-481.

(Chapter 2)

3. Effects of multiple rifampicin 450 mg doses on the fexofenadine enantiomers pharmacokinetics in Japanese volunteers

Akamine Y, Miura M, Yasui-Furukori N, Ieiri I, Uno T.
Manuscript in preparation

(Chapter 3)

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

Akamine Y, Miura M, Komori H, Saito S, Yamada S, Shiohira H, Kusuhara H, Tamai I, Ieiri I, Uno T, Yasui-Furukori N.
Manuscript in preparation

(Chapter 3)

List of Selected Relevant Publications

1. Effect of coadministration of single and multiple doses of rifampicin of fexofenadine enantiomers.
Kusuhara H, Miura M, Yasui-Furukori N, Yoshida K, Yokochi M, Fukizawa S, Ikejiri K, Kanamitsu K, Akamine Y, Uno T, sugiyama Y.
Drug Metab Dispos, 2013; 41, 206-213.
2. Psychotropic drug-drug interactions involving P-glycoprotein.
Akamine Y, Yasui-Furukori N, Ieiri I, Uno T.
CNS drugs 2012; 6, 959-973.
3. Different effects of the selective serotonin reuptake inhibitors fluvoxamine, paroxetine, and sertraline on the pharmacokinetics of fexofenadine in healthy volunteers.
Saruwatari J, Yasui-Furukori N, Niioka T, Akamine Y, Takashima A, Kaneko S, Uno T.
J Clin Psychopharmacol 2012; 32, 195-199.
4. The role of drug-transporters on psychotropic penetration at the blood-brain barrier.
Akamine Y, China K, Uno T.
Clinical Neuropsychopharmacology and Therapeutics 2012; 3, 8-14.
5. A sensitive column-switching HPLC method for aripiprazole and dehydroaripiprazole and its application to human pharmacokinetic studies.
Akamine Y, Yasui-Furukori N, Kojima M, Inoue Y, Uno T.
J Sep Sci 2010; 33, 3292-3298.
6. Effects of the P-glycoprotein inducer carbamazepine on fexofenadine pharmacokinetics.
Yamada S, Yasui-Furukori N, Akamine Y, Kaneko S, Uno T.
Ther Drug Monit 2009; 31, 764-768.

Chapter 1

Influence of Drug-Transporter Polymorphisms on the Pharmacokinetics of Fexofenadine Enantiomers

1.1. Introduction

P-glycoprotein (P-gp), encoded by *ABCB1* genes, is a membrane efflux transporter normally expressed in human tissues such as the small intestine, the biliary canalicular front of hepatocytes, and the renal proximal tubules [1]. A few studies have investigated whether *ABCB1* polymorphisms including 1236C>T, 2677G>A/T and 3435C>T mutations, could affect the pharmacokinetics of racemic fexofenadine and have yielded conflicting results [61,62]. Yi *et al.* have reported that the plasma concentrations of fexofenadine after a single oral administration of 180 mg fexofenadine were significantly lower in three subjects with *ABCB1* 2677AA/3435CC than in subjects with other genotypes (2677/3435: GG/CC, GT/CT, TT/TT, GA/CC, and TA/CT) [61]. On the other hand, Drescher *et al.* have reported that there were no statistically significant differences in the pharmacokinetic parameters of fexofenadine after single oral dose of 180 mg fexofenadine between subjects with 2677GG, GT and TT genotypes or 3435TT and CC genotypes [62].

In addition to P-gp, organic anion transporting-polypeptides (OATPs), encoded by *SLCO* genes, may also be relevant to fexofenadine pharmacokinetics [40]. Of the OATP drug-transporters, OATP1B1, 1B3 and 2B1 are reported to be involved in the hepatic uptake of fexofenadine [63-65], and although OATP1A2 and 2B1 are expressed in the small intestine, these transporters key intestinal uptake transporter for fexofenadine absorption [40,66]. In a single report, the area under the plasma concentration-time curve (AUC) of racemic fexofenadine was found to be significantly higher in two subjects with the *SLCO1B1* 521CC (*15/*15) genotype than in ten subjects with the TT genotype [67]. Therefore, studies of OATPs and P-gp imply that the pharmacokinetics of fexofenadine enantiomers may be affected

by genetic polymorphisms of these drug transporters.

It has also been reported that multidrug resistance protein 2 (MRP2), encoded by *ABCC2* genes, and breast cancer resistance protein (BCRP), encoded by *ABCG2*, may contribute to fexofenadine transport [52,53]. Fexofenadine is thought to be excreted into bile predominantly by MRP2 and to a minor extent by BCRP [52]. Therefore, genetic polymorphisms of MRP2 and BCRP may contribute to the stereoselective pharmacokinetics of fexofenadine enantiomers.

Thus, the intervention of multiple transporters in fexofenadine pharmacokinetics makes an investigation of its transport mechanisms difficult, and the effect of chirality on fexofenadine transport has not yet been addressed. Therefore, we needed to clarify the effect of *SLCO*, *ABCB1*, *ABCC2* and *ABCG2* genetic polymorphisms associated with pharmacokinetic differences for fexofenadine enantiomers.

1.2. Subjects and study design

Twenty-four healthy Japanese subjects (twelve males and twelve females) were enrolled in this study after giving informed written consent. Their mean age was 24.6 ± 3.7 years (range 22-36 years) and their mean weight was 57.4 ± 5.5 kg (range 46-65 kg). None of the subjects had a history of significant medical illness or drug hypersensitivity. All subjects were nonsmokers. None of the subjects had taken any drug for at least 1 week before or during the study. The study protocol was approved by the Ethics Committee of Hirosaki University School of Medicine.

Each subject received an oral dose of 60 mg of racemic fexofenadine (Allegra[®], Sanofi Aventis, Tokyo, Japan) with a glass of tap water at 9:00 A.M.. All subjects fasted for 10 hours before administration of fexofenadine and had a standard meal 4 hours after ingestion of fexofenadine. Beverages containing alcohol, caffeine, tea, or fruit juice were forbidden during the test period.

1.3. Results

Clinical pharmacokinetics of fexofenadine enantiomers

After a single oral dose of racemic fexofenadine (60 mg), the plasma and urine concentrations of fexofenadine enantiomers were measured over the course of 24 hours in twenty-four healthy subjects. The plasma concentration of (*R*)-fexofenadine at all time points was higher than those of the corresponding (*S*)-enantiomer (Fig. 2A). The AUC_{0-24} and the maximum plasma concentration (C_{max}) of (*R*)-fexofenadine were significantly greater than those of the (*S*)-enantiomer ($P < 0.001$, respectively). The *R/S* ratios for the fexofenadine AUC_{0-24} and C_{max} were 1.62 [95% confidence interval (CI) 1.49-1.76] and 1.39 (95% CI 1.25-1.54), respectively. The oral clearance (CL/F) of (*S*)-fexofenadine was significantly greater than that of (*R*)-fexofenadine ($P < 0.001$).

The amount of (*S*)-fexofenadine urinary excretion was slightly higher but not significantly different than that of (*R*)-fexofenadine (Fig. 2B). Renal clearance (CL_{renal}) of (*S*)-fexofenadine was significantly greater than that of (*R*)-fexofenadine ($P < 0.01$).

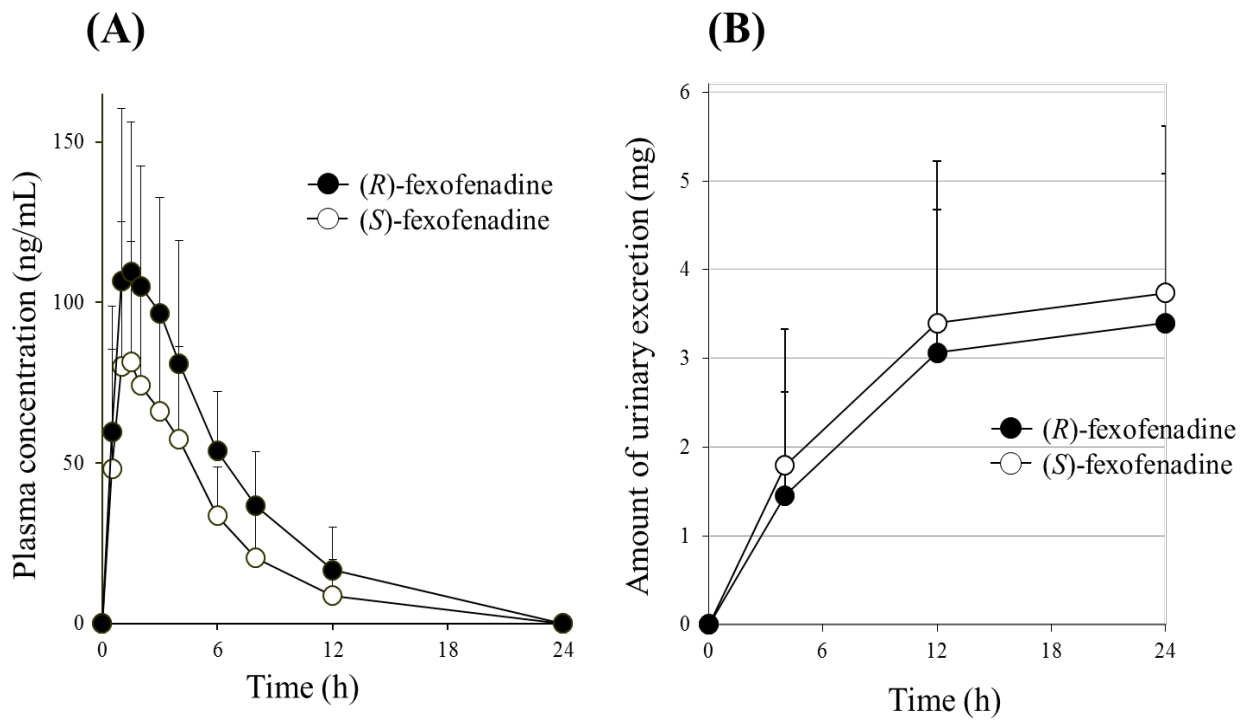


Fig. 2.

(A) Mean + SD of the plasma concentration-time profiles of (R)-fexofenadine (solid circles) and (S)-fexofenadine (open circles) after a 60 mg oral dose of racemic fexofenadine. (B) Mean + SD of the amount of urinary excretion of (R)-fexofenadine (solid circles) and (S)-fexofenadine (open circles) after a 60 mg oral dose of racemic fexofenadine. Time points consisted of 0, 6, 12, and 24 hours after fexofenadine administration.

Impacts of ABC genotype groups on the pharmacokinetics of fexofenadine enantiomers

There were no significant differences in the C_{\max} , elimination half-life ($t_{1/2}$), and AUC_{0-24} for (*R*)- and (*S*)-fexofenadine among the ABC polymorphisms including *ABCB1* 1236C>T, 2677G>A/T and 3435C>T, *ABCC2* -24C>T and *ABCG2* 421C>A (data not shown). On the other hand, the AUC_{0-24} and C_{\max} of (*S*)-fexofenadine were significantly lower in five subjects having a combination of *ABCB1* 1236CC/3435CC and *ABCC2* -24CC alleles than in subjects with other polymorphic genotypes ($P = 0.036$ and 0.015 , respectively), but no significant differences of the (*R*)-enantiomer were observed between the two groups (Fig. 3).

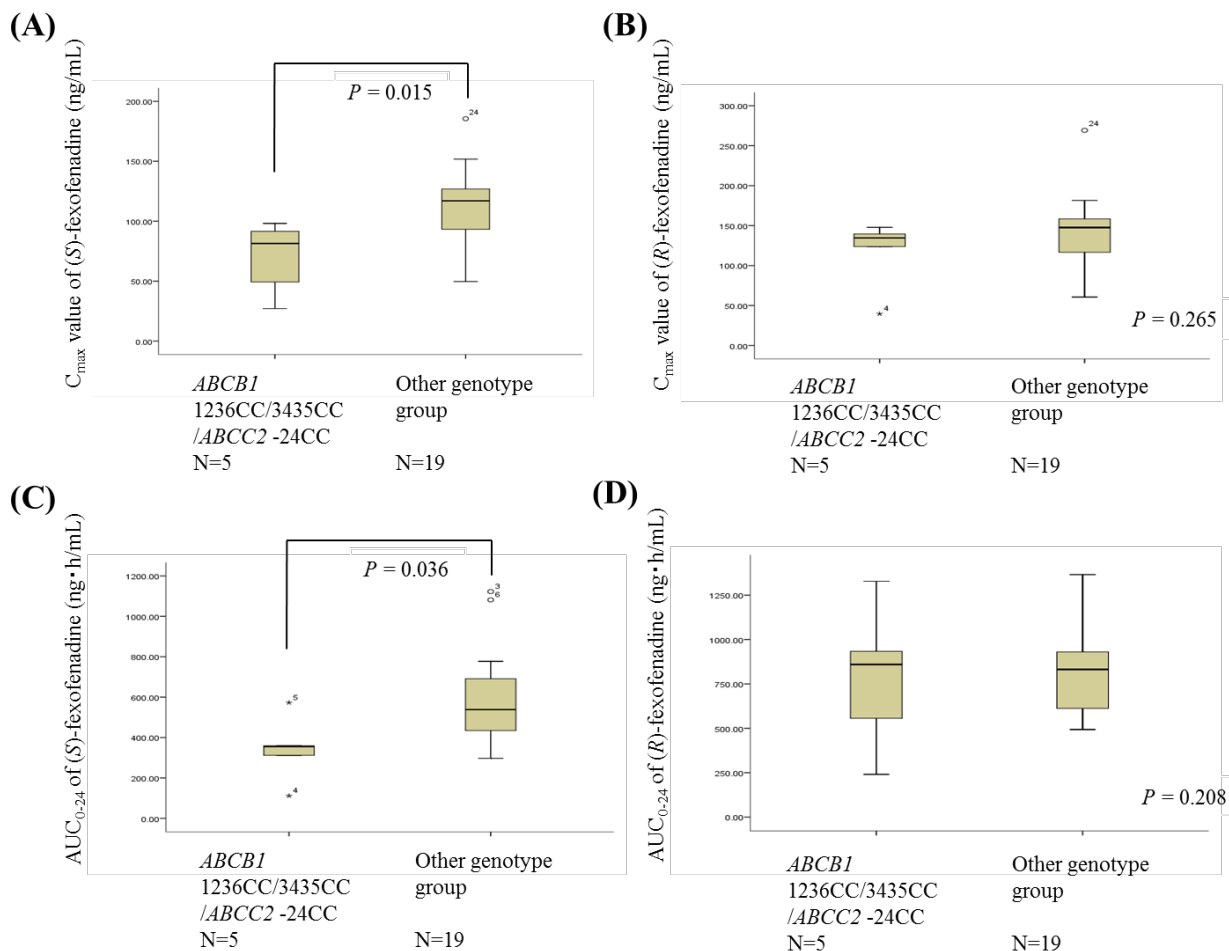


Fig. 3.

Comparison of the C_{max} and AUC_{0-24} of (S)-fexofenadine (A and C, respectively) and (R)-fexofenadine (B and D, respectively) between two genotype groups. Graphical analysis was done using the SPSS box and whiskers plot. The box spans data between the two quartiles (IQR), with the median represented as a bold horizontal line. The ends of the whiskers (vertical lines) represent the smallest and largest values that are not outliers. Outliers (circles) are values between 1.5 IQRs and 3 IQRs from the end of a box. Values greater than 3 IQRs from the end of a box are defined as extreme (asterisk). Other genotype group contains one or more polymorphism including *ABCB1* 1236CT or TT, *ABCB1* 3435CT or TT, *ABCC2* -24CT or TT.

Impact of OATPs genotype groups on the pharmacokinetics of fexofenadine enantiomers

The C_{\max} , $t_{1/2}$ and AUC_{0-24} of (*R*)- and (*S*)-fexofenadine in the OATPs genotype groups including *SLCO1B1*, *SLCO1B3* or *SLCO2B1* after a single oral administration of 60 mg racemic fexofenadine are shown in Table 1. The AUC_{0-24} of (*S*)-fexofenadine was significantly lower in fourteen subjects with the *SLCO2B1**1/*1 allele than in ten subjects with the *3 allele ($P = 0.031$).

Table 2 shows the pharmacokinetic parameters of fexofenadine enantiomers in the OATP2B1 plus P-gp or MRP2 genotype groups after a single oral administration of 60 mg racemic fexofenadine. The AUC_{0-24} and C_{\max} of (*S*)-fexofenadine were significantly lower in four subjects having a combination of *SLCO2B1**1/*1 and *ABCB1* 1236CC alleles than in subjects with other polymorphic genotypes ($P = 0.010$ and 0.029 , respectively), and the AUC_{0-24} of (*S*)-fexofenadine in subjects with the two combinations of *SLCO2B1**1/*1/*ABCB1* 3435CC and *SLCO2B1**1/*1/*ABCC2* -24CC was significantly lower than in other polymorphic genotype groups ($P = 0.033$ and 0.022 , respectively), while the AUC_{0-24} for (*R*)-fexofenadine in the combination *SLCO2B1**1/*1/*ABCB1* 1236CC genotype was significantly lower than in other polymorphic genotypes ($P = 0.045$).

In addition, there was no significant difference in all parameters for the (*R*)- and (*S*)-enantiomers between the OATP1B1 plus P-gp, MRP2 or BCRP genetic groups, and the OATP1B3 plus P-gp, MRP2 or BCRP genetic groups (data not shown). On the other hand, in the present study, we did not observe an influence of the *SLCO1A2* polymorphism on fexofenadine enantiomer pharmacokinetics, presumably since SNPs in *SLCO1A2* affecting transport activity have not been observed in an Asian population.

Table 1.

Pharmacokinetic parameters of fexofenadine enantiomers in each transporter genotype group after a single oral administration of 60 mg racemic fexofenadine.

	n	C _{max} (ng/mL)	P-values	t _{1/2} (h)	P-values	AUC ₀₋₂₄ (ng·h/mL)	P-values
(S)-fexofenadine							
<i>SLCO1B1</i>							
1a/1a+1a/1b+1b/1b	16	104 (27-186)	0.610 ^a	2.8 (1.8-4.9)	0.066 ^a	469 (112-1081)	0.569 ^a
1a/*15+1b/*15+*15/*15	8	122 (50-135)		3.6 (2.4-7.7)		546 (310-1123)	
<i>SLCO1B3</i>							
334T/T+T/G	13	122 (27-186)	0.569 ^b	3.3 (2.2-4.9)	0.320 ^b	519 (112-777)	1.000 ^b
334G/G	11	104 (49-152)		3.1 (1.8-7.7)		424 (298-1123)	
<i>SLCO2B1</i>							
*1/*1	14	111 (27-186)	0.931 ^b	2.6 (2.0-4.9)	0.120 ^b	446 (112-643)	0.031 ^b
*1/*3+*3/*3	10	113 (53-152)		3.6 (1.8-7.7)		675 (298-1123)	
(R)-fexofenadine							
<i>SLCO1B1</i>							
1a/1a+1a/1b+1b/1b	16	144 (40-269)	0.528 ^a	3.3 (2.5-5.7)	0.086 ^a	812 (241-1366)	0.928 ^a
1a/*15+1b/*15+*15/*15	8	136 (61-159)		4.5 (2.8-6.2)		848 (592-1004)	
<i>SLCO1B3</i>							
334T/T+T/G	13	138 (40-269)	0.494 ^b	3.7 (2.5-5.3)	0.769 ^b	832 (241-1328)	0.776 ^b
334G/G	11	140 (76-182)		3.5 (2.5-6.2)		860 (493-1366)	
<i>SLCO2B1</i>							
*1/*1	14	148 (40-269)	0.183 ^b	3.3 (2.5-5.7)	0.134 ^b	764 (241-1113)	0.212 ^b
*1/*3+*3/*3	10	133 (61-179)		4.0 (2.5-6.2)		916 (496-1366)	

The values shown are the median (range).

^a Kruskal-Wallis test.

^b Mann-Whitney *U* test vs. wild-type.

Table 2.

Pharmacokinetic parameters of fexofenadine enantiomers in each transporter genotype group after a single oral administration of 60 mg racemic fexofenadine.

	n	C _{max} (ng/mL)	P-values	t _{1/2} (h)	P-values	AUC ₀₋₂₄ (ng•h/mL)	P-values
(S)-fexofenadine							
<i>SLCO2B1/ABCB1</i> C1236T							
<i>SLCO2B1</i> *1/*1/ <i>ABCB1</i> 1236CC	4	67 (27-98)	0.029	2.1 (2.0-3.4)	0.097	334 (112-359)	0.010
other	20	108 (50-186)		3.2 (1.8-7.7)		556 (298-1123)	
<i>SLCO2B1/ABCB1</i> C3435T							
<i>SLCO2B1</i> *1/*1/ <i>ABCB1</i> 3435CC	6	74 (27-129)	0.090	2.4 (2.0-4.9)	0.343	357 (112-519)	0.033
other	18	109 (50-186)		3.2 (1.8-7.7)		579 (298-1123)	
<i>SLCO2B1/ABCC2</i> C-24T							
<i>SLCO2B1</i> *1/*1/ <i>ABCC2</i> -24CC	11	89 (27-135)	0.228	2.9 (2.0-4.9)	0.733	359 (112-643)	0.022
other	13	112 (53-186)		3.3 (1.8-7.7)		610 (298-1123)	
<i>SLCO2B1/ABCG2</i> C421A							
<i>SLCO2B1</i> *1/*1/ <i>ABCG2</i> 421CC	6	92 (27-135)	0.581	2.3 (2.0-4.9)	0.224	446 (112-643)	0.224
other	18	105 (50-186)		3.3 (1.8-7.7)		576 (298-1123)	
(R)-fexofenadine							
<i>SLCO2B1/ABCB1</i> C1236T							
<i>SLCO2B1</i> *1/*1/ <i>ABCB1</i> 1236CC	4	115 (40-148)	0.431	2.9 (2.7-4.1)	0.157	556 (241-860)	0.045
other	20	140 (61-269)		3.8 (2.5-6.2)		870 (493-1366)	
<i>SLCO2B1/ABCB1</i> C3435T							
<i>SLCO2B1</i> *1/*1/ <i>ABCB1</i> 3435CC	6	120 (40-159)	0.537	3.4 (2.7-4.9)	0.454	681 (241-877)	0.090
other	18	142 (61-269)		3.7 (2.5-6.2)		880 (493-1366)	
<i>SLCO2B1/ABCC2</i> C-24T							
<i>SLCO2B1</i> *1/*1/ <i>ABCC2</i> -24CC	11	126 (40-182)	0.494	3.5 (2.7-5.7)	0.459	764 (241-1032)	0.063
other	13	145 (61-269)		3.9 (2.5-6.2)		902 (493-1366)	
<i>SLCO2B1/ABCG2</i> C421A							
<i>SLCO2B1</i> *1/*1/ <i>ABCG2</i> 421CC	6	130 (40-182)	0.626	2.8 (2.7-4.9)	0.122	764 (241-1032)	0.199
other	18	138 (61-269)		3.9 (2.5-6.2)		855 (493-1366)	

The values shown are the median (range).

Mann-Whitney *U* test vs.wild-type.

1.4. Discussion

This is the first report investigating the association of drug-transporter polymorphisms with fexofenadine enantiomers pharmacokinetics. In this study, the OATP2B1 polymorphism is a key determinant of (*S*)-fexofenadine pharmacokinetics, and polymorphisms of P-gp and MRP2 in addition to OATP2B1 were associated with altered plasma concentrations of (*S*)-fexofenadine after a single oral administration of 60 mg fexofenadine.

In our previous papers, we suggested that P-gp could play a key role in the differences of fexofenadine enantiomer pharmacokinetics [59,60]. However, in the present study, other transporters besides P-gp were observed to affect fexofenadine enantiomer pharmacokinetics. This result suggests that P-gp genetic polymorphisms are somewhat less important factors in determining the stereoselectivity of fexofenadine enantiomers. Previous papers have reported that the co-administration of itraconazole and verapamil decreased the mean *R/S* ratio for the fexofenadine AUC from 1.84 to 1.43 and from 1.76 to 1.32, respectively [59,60]. An *R/S* ratio not around 1.00 implies that the difference between fexofenadine enantiomers cannot be completely explained only on the basis of chiral discrimination by P-gp and suggests the involvement of multiple drug-transporters.

In the present study, only the OATP2B1 genetic polymorphism was associated with fexofenadine enantiomer pharmacokinetics, and the AUC₀₋₂₄ of (*S*)-fexofenadine was significantly lower in subjects with the *SLCO2B1**1/*1 allele than in those with the *3 allele ($P = 0.031$) (Table 1); however, there were no significant differences in the AUC₀₋₂₄ of (*R*)-fexofenadine between the *SLCO2B1* genotypes. This finding suggests that OATP2B1 contributes more to the transport of

(*S*)-fexofenadine than of the (*R*)-enantiomer. In addition, *SLCO2B1**3 allele decreases the transport function of OATP2B1 [68]. A decrease in the function of OATP2B1 in the small intestine, if any, ought to cause a reduction in intestinal uptake and a resultant decrease in plasma concentrations of both enantiomers. Thus, these results support that (*S*)-fexofenadine transport by OATP2B1 may be greater in the liver than in the small intestine because OATP2B1 expression is most abundant in human liver [63], and the influence of the OATP2B1 polymorphism on (*S*)-fexofenadine transport in the small intestine may be minimal. Therefore, hepatic uptake of fexofenadine may be enantioselective and the subsequent hepato-biliary transport would be enantioselective irrespective of biliary excretion selectivity.

In the present study as analyzed by the pharmacokinetic parameters in OATP2B1 plus P-gp or MRP2 genotype groups, the AUC₀₋₂₄ of (*S*)-fexofenadine was significantly lower in subjects with the triple combination of *SLCO2B1**1/*1/*ABCB1* 1236CC, *SLCO2B1**1/*1/*ABCB1* 3435CC, and *SLCO2B1**1/*1/*ABCC2* -24CC than in subjects with other polymorphic genotype groups, while the AUC₀₋₂₄ of (*R*)-fexofenadine was significantly lower in subjects with a combination of *SLCO2B1**1/*1/*ABCB1* 1236CC than in subjects with other polymorphic genotypes (Table 2). These findings suggest that differences of fexofenadine enantiomer pharmacokinetics may be affected by combinations of OATP2B1, P-gp, and MRP2 genetic polymorphisms. Furthermore, mean AUC₀₋₂₄ and C_{max} values of (*S*)-fexofenadine were significantly lower in subjects with the *ABCB1* 1236CC/3435CC/*ABCC2* -24CC genotypes than with other genotype groups ($P = 0.036$ and 0.015 , respectively) (Fig. 3), but there was no significant difference in the $t_{1/2}$ of (*S*)-fexofenadine between the two groups ($P = 0.367$). This finding shows that the oral absorption of (*S*)-fexofenadine is mainly influenced by the

intestinal expression of P-gp and MRP2 based on their genetic polymorphisms.

The regulation by transporters such as P-gp, OATPs, MRP2, and minorly BCRP for fexofenadine exposure is complex; therefore, we may not find definitive transporter polymorphisms for (*R*)-fexofenadine. Other single-nucleotide polymorphisms in addition to the transporter polymorphisms observed in the present study may influence the pharmacokinetics of (*R*)-fexofenadine. This study was carried out in a small clinical trial with only twenty-four healthy Japanese subjects; hence, further study using a larger sample size is necessary, and our results should be interpreted within the context of the study limitations.

1.5. Conclusion

In conclusion, the pharmacokinetics of (*S*)-fexofenadine is associated with a single polymorphism of *SLCO2B1*, and combinations of several polymorphisms of *ABCB1* C1236T, C3435T and *ABCC2* C-24T. Our findings suggest that the combination of multiple transporters involving OATPs, P-gp, and MRP2 reacts strongly to fexofenadine exposure in the small intestine and liver, resulting in different disposition between both enantiomers.

Chapter 2

The Role of P-glycoprotein on the Pharmacokinetics of Fexofenadine Enantiomers

2.1. Introduction

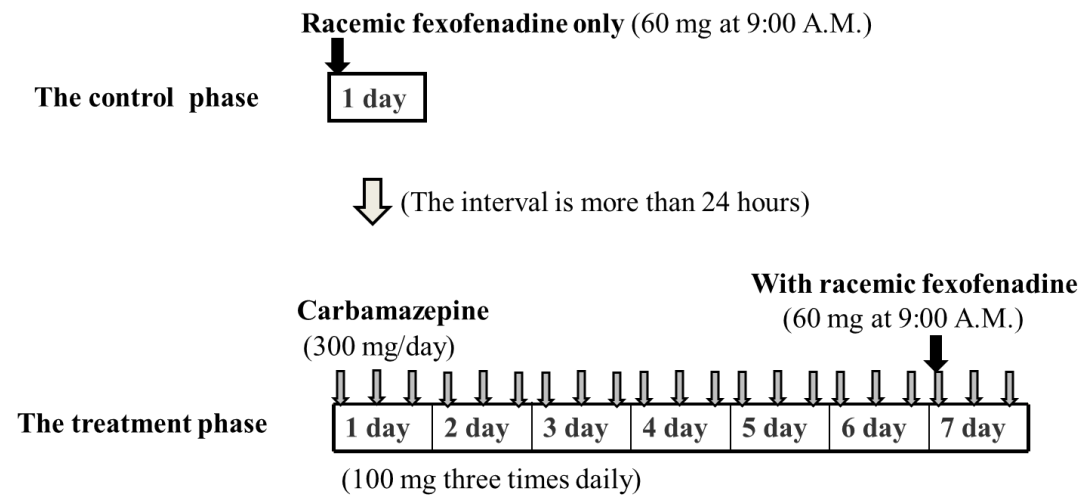
Recently, we have reported that itraconazole or verapamil co-administration altered the plasma concentrations of (*R*)- and (*S*)-fexofenadine enantiomers through the probable inhibition of P-gp-mediated transport [59,60]. Because the C_{max} and the plasma concentration at the first sample point of both enantiomers were increased, these findings imply that the P-gp-mediated transport of fexofenadine may be primarily inhibited by P-gp inhibitors in the small intestine. In the first chapter, *ABCB1* polymorphisms were associated with altered plasma concentrations of (*S*)-fexofenadine after a single oral administration of 60 mg fexofenadine.

Meanwhile, carbamazepine is known to be a potent CYP3A inducer, several *in vitro* and *in vivo* reports regarding drug-drug interactions have shown that carbamazepine is also a P-gp inducer [69] that can markedly reduce plasma concentrations and the efficacy of talinolol as a P-gp substrate [70]. Therefore, if the stereoselective disposition of fexofenadine is caused by P-gp-mediated transport, carbamazepine may alter the different properties of each fexofenadine enantiomer. To date, no information is available to suggest an *in vivo* contribution of a P-gp inducer in the stereoselective effects of racemic mixtures. Therefore, the principal aim was to evaluate the possible effects of the P-gp inducer carbamazepine on fexofenadine enantiomer pharmacokinetics in healthy volunteers. The present results may indicate how the stereoselectivity of fexofenadine will be changed by P-gp inducers and will lead to further research concerning the chirality of racemic mixtures.

2.2. Subjects and study design

Twelve healthy Japanese volunteers (males) were enrolled in this study after giving informed written consent. 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 mean (\pm SD) age and body weight of the volunteers were 25.2 (\pm 5.9) years (range 20–39 years) and 62.4 (\pm 4.1) kg (range 58–70 kg), respectively. This study was approved by the Ethics Committee of the Hirosaki University School of Medicine. This randomized, open-label study consisted of two phases (a control and a 7-day treatment) and 2 study days in which 60 mg of fexofenadine hydrochloride was administered (Fig. 4). In the control phase, volunteers received 60 mg of fexofenadine hydrochloride (Allegra[®]; Sanofi-Aventis K.K., Tokyo, Japan) at 9:00 A.M. after an overnight fast. In the treatment phase, carbamazepine was dosed at 100 mg 3 times daily (for a total daily dose of 300 mg) for 7 days. On day 7, a single 60 mg dose of fexofenadine was co-administered with a 100 mg dose of carbamazepine (Tegretol[®]; Novartis Pharma Ltd., Tokyo, Japan) at 9:00 A.M. after an overnight fast. In a second volunteer group, fexofenadine was administered alone after a 2-week washout period. The order of the two phases was randomly assigned to each volunteer. Six volunteers started the control phase, which was first followed by the treatment phase more than 24 hours after the last blood sampling in the control phase (Fig. 4A). The other volunteers started the treatment phase, which was first followed by a treatment hiatus of at least 2 weeks and the control phase (Fig. 4B). Volunteers did not take any medication or fruit juices for at least 7 days before both study phases, and no meal or beverages were allowed until 4 hours after fexofenadine administration.

(A)



(B)

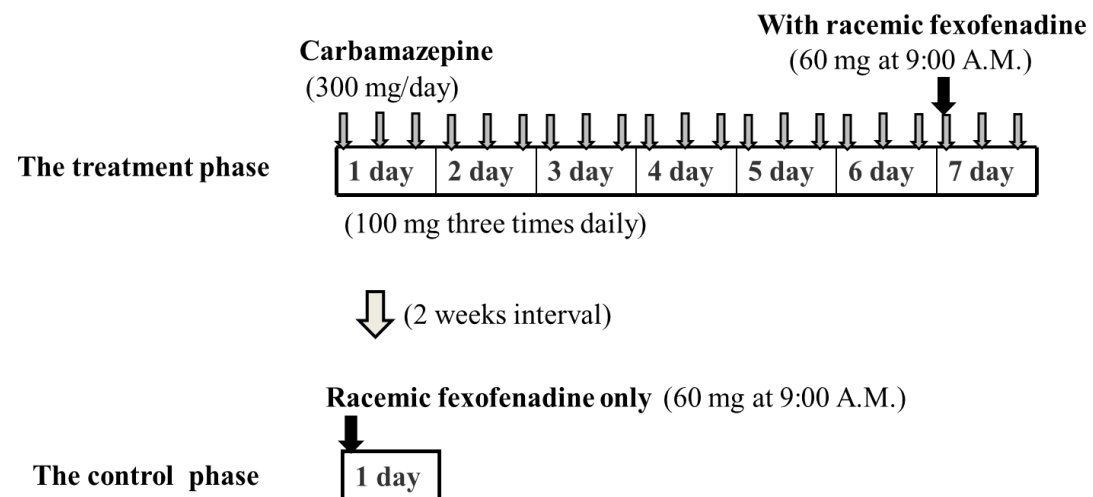


Fig. 4. Study design.

(A) Six volunteers started the control phase, which was first followed by the treatment phase more than 24 hours after the last blood sampling in the control phase. (B) Six volunteers started the treatment phase, which was first followed by a treatment hiatus of at least 2 weeks and the control phase. The order of the two phases was randomly assigned to each volunteer.

2.3. Results

Effects of the carbamazepine on the plasma concentrations of fexofenadine enantiomers

Four subjects in the carbamazepine phase experienced somnolence, which is consistent with known reactions to carbamazepine; however, these drug-related adverse events were mild in intensity, and the subjects completed all phases according to the study protocol.

The mean (+ SD) plasma concentration-time profiles of the fexofenadine enantiomers in both phases are shown in Fig. 5, and the pharmacokinetic parameters are summarized in Table 3. In the control phase, the plasma concentration of (*R*)-fexofenadine at all time points was higher than the corresponding (*S*)-enantiomer, and the mean AUC₀₋₂₄ *R/S* ratio was 1.58 (95% CI 1.48-1.68) (Table 3). Similar to the results of our previous reports [56,57,59,60], the mean AUC₀₋₂₄ ($P < 0.001$) and C_{max} ($P < 0.05$) of (*R*)-fexofenadine were significantly greater than the (*S*)-enantiomer. The mean CL/F ($P < 0.001$) of (*S*)-fexofenadine was significantly greater than that of (*R*)-fexofenadine (Table 3).

Carbamazepine co-administration significantly decreased plasma concentrations of both fexofenadine enantiomers at the final sample point from the initial sample point in comparison to the enantiomers that were measured during the control phase (Fig. 5) and altered all pharmacokinetic parameters except the time to reach C_{max} (t_{max}) (Table 3). Although the mean AUC₀₋₂₄ ($P < 0.001$ for (*S*)-fexofenadine, $P < 0.001$ for (*R*)-fexofenadine, respectively) values of both enantiomers were significantly decreased in the carbamazepine phase, the mean individual differences between the control and carbamazepine phases for the AUC₀₋₂₄ ($P < 0.001$) of

(*S*)-fexofenadine were significantly greater than those of (*R*)-fexofenadine (Table 3). In addition, although the $t_{1/2}$ values were not different between the (*R*)- and (*S*)-enantiomers in the control phase ($P = 0.231$), carbamazepine significantly shortened the mean $t_{1/2}$ ($P < 0.05$) of (*S*)-fexofenadine without affecting (*R*)-fexofenadine (Table 3). There were significant differences in the $t_{1/2}$ ($P < 0.001$) between both enantiomers in the carbamazepine phases (Table 3).

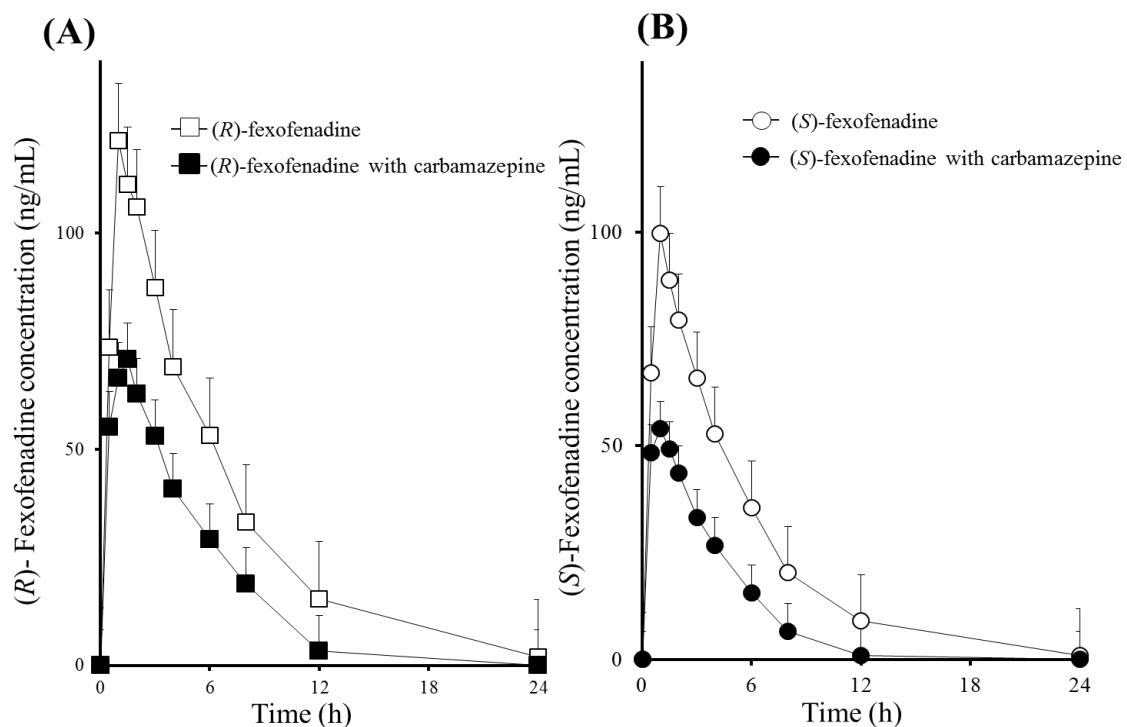


Fig. 5. (A) Mean (+ SD) plasma concentration–time curves of (*R*)-fexofenadine following a single oral administration of 60 mg fexofenadine hydrochloride in twelve healthy volunteers treated with placebo (open squares) or carbamazepine (closed squares). (B) Mean (+ SD) plasma concentration–time curves of (*S*)-fexofenadine following a single oral administration of 60mg fexofenadine hydrochloride in twelve healthy volunteers treated with placebo (open circles) or carbamazepine (closed circles).

Effect of the carbamazepine on the urinary excretion of fexofenadine enantiomers

In contrast to the ratios of (*R*)- and (*S*)-fexofenadine plasma concentrations, the urine concentration of (*S*)-fexofenadine was slightly higher than that of (*R*)-fexofenadine in the control phase (Fig. 6); the pharmacokinetic parameters are summarized in Table 3. In the control phase, there were significant differences in the mean CL_{renal} ($P < 0.01$) of both enantiomers; however, the mean Ae_{0-24} ($P = 0.541$) values were not different between the (*R*)- and (*S*)-enantiomers (Table 3).

Similar to the results of the plasma concentrations, carbamazepine co-administration significantly decreased the urine concentrations of both enantiomers (Fig. 6), and the mean Ae_{0-24} values of both enantiomers were significantly decreased in the carbamazepine phase ($P < 0.01$ for (*R*)-fexofenadine, $P < 0.05$ for (*S*)-fexofenadine, respectively). Although the CL_{renal} ($P = 0.154$) of (*R*)-fexofenadine did not change between the control and carbamazepine phases, that of (*S*)-fexofenadine ($P < 0.01$) was significantly increased in the carbamazepine phases in most of the volunteers (Table 3). In addition, the mean individual differences in the CL_{renal} ($P < 0.001$) of (*S*)-fexofenadine between the control and carbamazepine phases were significantly greater than those of (*R*)-fexofenadine. The mean CL_{renal} *R/S* ratio of 0.64 (95% CI 0.51-0.77) in the control phase decreased significantly to 0.46 (95% CI 0.41-0.50) in the carbamazepine phase ($P < 0.001$) (Table 3).

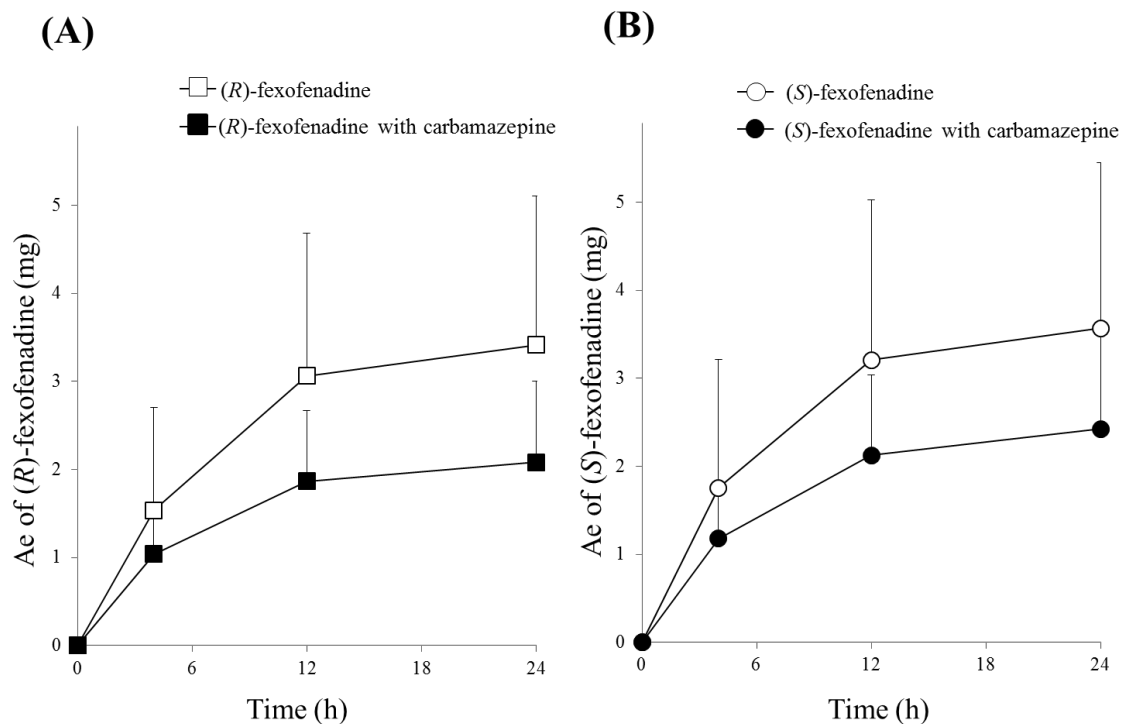


Fig. 6.

(A) Mean (+ SD) cumulative amount of (*R*)-fexofenadine excreted into urine following a single oral administration of 60 mg fexofenadine hydrochloride in twelve healthy volunteers treated with placebo (open squares) or carbamazepine (closed squares). (B) Mean (+ SD) cumulative amount of (*S*)-fexofenadine excreted into urine following a single oral administration of 60mg fexofenadine hydrochloride in twelve healthy volunteers treated with placebo (open circles) or carbamazepine (closed circles).

Table 3.

Effect of carbamazepine on pharmacokinetic parameters of fexofenadine enantiomers

Parameters	With control	With carbamazepine	Ratio to control
(R)-fexofenadine			
$t_{1/2}$ (h)	4.2 (3.4-4.9)	3.3 (2.8-3.9) ^{†††}	0.84 (0.71-0.96)
t_{max} (h)(range)	1.4 (1.1-1.7)	1.1 (0.8-1.5)	0.80 (0.62-1.05)
C_{max} (ng/mL)	132 (103-161) [†]	85 (64-107) ^{** †††}	0.68 (0.53-0.82)
AUC_{0-24} (ng·h/mL)	749 (656-842) ^{†††}	359(303-415) ^{*** †††}	0.49 (0.40-0.57)
CL/F (L/h)	42 (36-48) ^{†††}	86 (63-108) ^{*** †††}	2.00 (1.90-2.45)
Ae_{0-24} (mg)	3.4 (2.5-4.4)	2.1(1.6-2.6) ^{** †††}	0.69 (0.47-0.92)
CL_{renal} (L/h)	4.7 (3.3-6.0) ^{††}	6.1(4.5-7.7) ^{†††}	1.62 (0.89-2.36)
(S)-fexofenadine			
$t_{1/2}$ (h)	3.7 (2.7-4.7)	2.5 (2.1-2.8) [*]	0.76 (0.57-0.97)
t_{max} (h)(range)	1.5 (1.0-2.0)	1.1 (0.7-1.4)	0.81 (0.56-1.05)
C_{max} (ng/mL)	100 (83-118)	68 (47-88) [*]	0.69 (0.49-0.88)
AUC_{0-24} (ng·h/mL)	481 (410-552)	187 (153-222) ^{***}	0.40 (0.31-0.49)
CL/F (L/h)	67 (56-77)	174 (145-202) ^{***}	2.71 (2.28-3.13)
Ae_{0-24} (mg)	3.6 (2.5-4.6)	2.4 (1.8-3.0) [*]	0.79 (0.54-1.05)
CL_{renal} (L/h)	7.5 (5.5-9.6)	13.6 (10.0-17.3) ^{**}	2.20 (1.31-3.09)
R/S ratio of AUC_{0-24}	1.58 (1.48-1.68)	1.93 (1.78-2.09) ^{**}	1.24 (1.11-1.37)
R/S ratio of CL_{renal}	0.64 (0.51-0.77)	0.46 (0.41-0.50) ^{***}	0.76 (0.66-0.86)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, between control phase and carbamazepine phase.

† $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$, between (R)-fexofenadine and (S)-fexofenadine.

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

2.4. Discussion

We investigated the effects of the P-gp inducer carbamazepine on the pharmacokinetics of fexofenadine enantiomers. Similar to the results of previous reports [56,57,59,60], the present study demonstrated that the plasma concentration of (*R*)-fexofenadine was higher than the corresponding (*S*)-enantiomer during the control phase, and the stereoselectivity was altered by carbamazepine treatment (Fig. 5). Carbamazepine significantly decreased the mean AUC₀₋₂₄ of both enantiomers, but this effect was greater for (*S*)-fexofenadine, resulting in a mean increase in AUC₀₋₂₄ *R/S* ratio from 1.58 to 1.93 ($P < 0.01$) (Table 3). Previous *in vitro* studies have suggested that P-gp plays a major role in the efflux of fexofenadine in the small intestine, whereas it has a limited role in biliary excretion [53]. Therefore, the present result may suggest that the P-gp-inductive effect of carbamazepine in the small intestine could result in a decrease of the mean AUC₀₋₂₄ of both enantiomers and these different effects may be due to the affinity of P-gp for each enantiomer.

In addition, although the $t_{1/2}$ values were not different between the (*R*)- and (*S*)-enantiomers in the control phase, there were significant differences in the $t_{1/2}$ between both enantiomers in the carbamazepine phases (Table 3). These results may suggest that the present findings of decreasing the $t_{1/2}$ is due to the combinative induction of the intestinal- and hepatic-efflux transporters including P-gp by carbamazepine, because about two-thirds of bioavailable fexofenadine is estimated to be excreted into bile.

Interestingly, the mean *R/S* ratio of AUC₀₋₂₄ did not approach 1.00 in the carbamazepine phases, implying that a carbamazepine dose of 300 mg may be insufficient to achieve substantial inductive effects of P-gp-mediated transport.

Furthermore, in the present study, although we did not measure carbamazepine concentration, an assessment of the relationship between plasma carbamazepine concentration and fexofenadine pharmacokinetics would be more informative.

Alternatively, the enantioselective disposition of fexofenadine may not be completely explained solely on the basis of chiral discrimination by P-gp, because fexofenadine is also a substrate of other drug transporters, including OATPs and MRP2. Consequently, the present results suggest that these drug transporters might play roles in the stereoselective pharmacokinetics of fexofenadine [40]. Additionally, although carbamazepine is also known to be an MRP2 inducer [70], little is known about whether carbamazepine is a substrate or an inducer of OATPs. Therefore, the different effects of carbamazepine on the pharmacokinetics of fexofenadine enantiomers may be partially attributed to MRP2, in addition to P-gp.

In the first chapter, we indicated that *SLCO* (the gene encoding OATP) polymorphisms strongly associated with the pharmacokinetics of fexofenadine enantiomers. The pharmacokinetics of (*S*)-fexofenadine are affected by a polymorphism of *SLCO2B1* in the first chapter. Therefore, these results suggest that OATP2B1 plays an important role in (*S*)-fexofenadine pharmacokinetics. Our findings suggest that a combination of multiple transporters, including OATP2B1, P-gp, and MRP2, may be strongly influenced by fexofenadine exposure and result in different dispositions between the enantiomers.

2.5. Conclusion

In conclusion, this study indicates that intestinal P-gp is a key determinant for the stereoselective pharmacokinetics of fexofenadine, and such stereoselectivity is altered by carbamazepine, a recognized inducer of P-gp. In addition, because the inductive effect of carbamazepine to P-gp may be different between the fexofenadine enantiomers can not eliminate, it is likely that other transporters, including OATP2B1 and MRP2, also contribute to the stereoselective pharmacokinetics of fexofenadine.

Chapter 3

The Role of Organic Anion Transporting Polypeptides on the Pharmacokinetics of Fexofenadine Enantiomers

3.1. Effects of multiple rifampicin 450 mg doses on the pharmacokinetics of fexofenadine enantiomers

3.1.1. Introduction

Rifampicin is a potent inducer of the CYP enzyme system and the P-gp transport system, and it markedly reduces the plasma concentrations and the efficacy of these substrate drugs [71,72]. Moreover, since CYP3A substrates considerably overlapped with P-gp substrates, the inductive effects by rifampicin may be occurred through the combination of CYP3A and P-gp [73]. However, recent *in vivo* studies have shown that rifampicin produces an increase in the exposure to several drugs [16] because rifampicin inhibits 4 types of OATPs i.e., OATP1A2, 1B1, 1B3 and 2B1, in both the gut and liver at several *in vitro* studies [2,17]. In a recent study, a single dose of rifampicin significantly increases both the C_{max} and the total AUC of atorvastatin [74]. Although atorvastatin is a substrate of CYP3A, P-gp and OATPs [74,75], this result indicates that rifampicin may be inhibited the OATPs-mediated hepatic uptake of atorvastatin because the OATPs-inhibition of the intestinal uptake decreases the concentrations of OATPs substrates as shown by fruit juices studies [18,76]. Consistent with this finding, further clinical studies have also shown that a single dose of rifampicin increases the plasma concentration of several OATPs substrate drugs, such as atrasentan, bosentan, glyburide and repaglinide [12-15]. Consequently, these findings are consistent with several *in vitro* reports [17,77] and suggest that rifampicin is a potent OATPs inhibitor whose effects may be greater on the hepatic uptake than the intestinal uptake.

In multiple-dose rifampicin studies, interactions between rifampicin and OATPs substrates are caused by various factors. Both ambrisentan and atorvastatin are

substrates of CYP3A, P-gp and OATPs, and multiple doses of rifampicin have no effects on the AUC of ambrisentan [78] but markedly reduce the AUC of atorvastatin [79]. These different influences may be due primarily to the involvement of CYPs and P-gp induction in drug interactions, and followed the extent of OATPs inhibition [16]. In addition, pitavastatin is a substrate of OATPs and P-gp but not CYP3A4, and the AUC is significantly increased to 1.3-fold by multiple doses of rifampicin [80]. This result may be greater in the hepatic OATPs-inhibition than the P-gp induction. In addition to these potential OATPs inhibition, these findings may imply that the multiple-dose rifampicin induces OATPs-mediated transport on biliary and kidney elimination in addition to intestinal absorption [16,71]. Therefore, because of the inhibitory and/or inductive effects of multiple-dose rifampicin on the transports and metabolisms, complex drug-drug interactions have been observed between rifampicin and these substrate drugs.

The first chapter indicated that *SLCO* (encoding OATP) polymorphisms are more associated with the pharmacokinetics of fexofenadine enantiomers than *ABCB1* (also MDR1 encoding P-gp) polymorphisms. In addition, single and multiple 600 mg doses of rifampicin significantly increase the concentrations of both enantiomers through the probable inhibition of the OATPs transporters [43]. However, although this study [43] and other previous OATPs-interactions reports used rifampicin 600 mg dose [12-15,74], there is no information of the effect by a simultaneous and clinical doses (450 mg) of rifampicin well used by Japanese patients.

Therefore, the principal aim of the present study was to evaluate the possible effects of multiple 450 mg doses of rifampicin on fexofenadine enantiomer

pharmacokinetics in Japanese healthy volunteers. Subsequently, by comparing both the P-gp-inductive and the OATPs-inhibited effects after rifampicin dosing, we examined which drug transporters contributed to the stereoselectivity of fexofenadine pharmacokinetics.

3.1.2. Subjects and study design

Ten healthy Japanese volunteers (seven males and three females) were enrolled in this study after giving informed written consent. 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 mean (\pm SD) age and body weight of the volunteers were 26.1 (\pm 6.0) years (range 21–39 years) and 60.5 (\pm 14.3) kg (range 44–95 kg), respectively. This study was approved by the Ethics Committee of the Hirosaki University School of Medicine.

A randomized, double-blinded placebo-controlled cross-over study design with two phases (a control and a 7-day treatment) was used with an interval of 4 weeks (Fig. 7). Ten healthy volunteers received either 450 mg of rifampicin in capsule form (three 150 mg rifampicin capsules, Rifadin[®], Daiichi-Sankyo Pharmaceutical, Tokyo, Japan) or a matched placebo in capsule form with the same appearance and size as rifampicin orally once daily at 9:00 A.M. for 7 days. On day 7, a single 60 mg dose of racemic fexofenadine hydrochloride (Allegra[®], Sanofi-Aventis K.K., Tokyo, Japan) was co-administered with 200 mL water at 9:00 A.M. after an overnight fast. Volunteers did not take any medication or fruit juices for at least 7 days before both study phases, and no meals or beverages were allowed until 4 hours after racemic-fexofenadine administration.

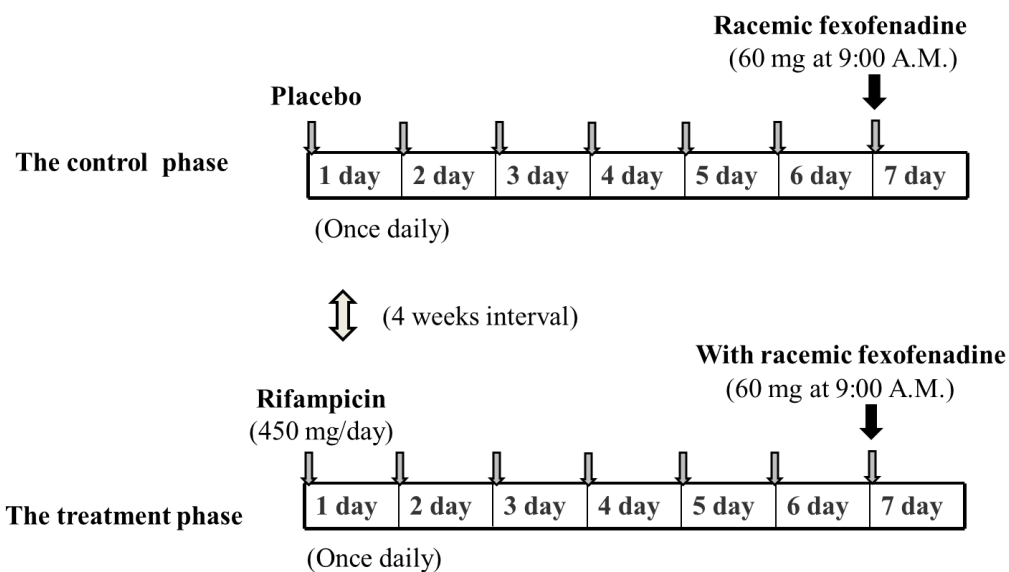


Fig. 7. Study design.

Ten healthy volunteers received either 450 mg of rifampicin in capsule form or a matched placebo in capsule form with the same appearance and size as rifampicin orally once daily. The order of the two phases was randomly assigned to each volunteer.

3.1.3. Results

Effect of the rifampicin on the plasma concentrations of fexofenadine enantiomers

None of the enrolled subjects reported any adverse events during the study, and they completed all phases according to the study protocol.

The mean (+ SD) plasma concentration-time profiles of the fexofenadine enantiomers after a single oral administration of 60 mg fexofenadine hydrochloride in both the control and rifampicin-treated phases are shown in Fig. 8, and the pharmacokinetic parameters are summarized in Table 4. In the control phase, the mean plasma concentrations of (*R*)-fexofenadine were higher than those of the (*S*)-enantiomer (Fig. 8). Similar to our previous results [56,57,59,60], the mean AUC_{0-24} ($P < 0.01$) and C_{max} ($P < 0.001$) of (*R*)-fexofenadine were greater than those of the (*S*)-enantiomer (Table 4). The mean AUC_{0-24} *R/S* ratio was 1.54 (95% CI, 1.38-1.73) (Table 4).

Rifampicin co-administration markedly raised the plasma concentrations of both enantiomers at the final sample point from the initial sample point compared to the enantiomers that were measured during the control phase (Fig. 8). Rifampicin significantly altered the pharmacokinetic parameters, except for the $t_{1/2}$ and t_{max} , of both enantiomers (Table 4). Although rifampicin strongly elevated the mean AUC_{0-24} values of both enantiomers ($P < 0.01$ for both enantiomers), the mean individual differences between the control and rifampicin phases for the AUC_{0-24} of (*S*)-fexofenadine were greater than those of (*R*)-fexofenadine ($P < 0.01$) (Fig. 10A). Rifampicin decreased the mean AUC_{0-24} *R/S* ratio from 1.54 to 1.39 (95% CI, 1.30-1.48), but this difference was not significant (Table 4). Although there was no significant difference in the mean $t_{1/2}$ between the (*R*)- and (*S*)-enantiomers in the

control phase, the mean $t_{1/2}$ of the (*S*)-enantiomer was shortened in the rifampicin phase ($P < 0.01$) (Table 4).

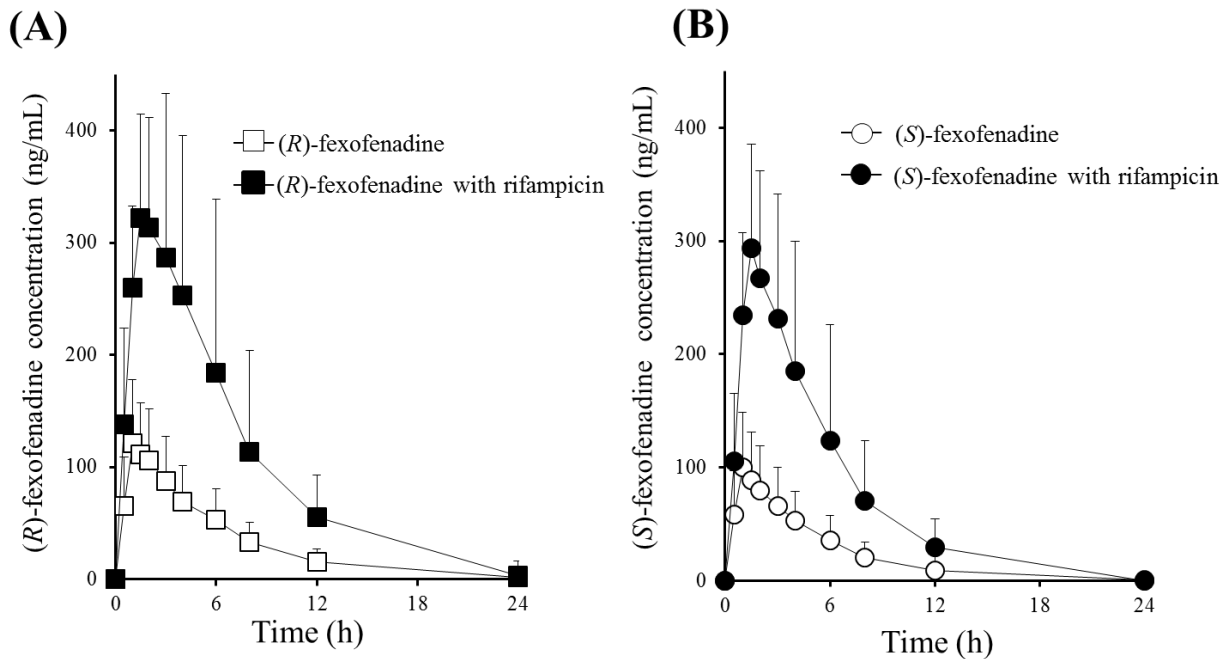


Fig. 8. (A) Mean (+SD) plasma concentration–time curves of (*R*)-fexofenadine following a single oral administration of 60 mg fexofenadine hydrochloride in ten healthy volunteers treated with placebo (open squares) or rifampicin (closed squares). (B) Mean (+SD) plasma concentration–time curves of (*S*)-fexofenadine following a single oral administration of 60 mg fexofenadine hydrochloride in ten healthy volunteers treated with placebo (open circles) or rifampicin (closed circles).

Effect of the rifampicin on the urinary excretion of fexofenadine enantiomers

The time profile means (+ SD) Ae_{0-24} of fexofenadine enantiomers in both phases are shown in Fig. 9, and the urine pharmacokinetic parameters are summarized in Table 4. In contrast to the majority of (*R*)-fexofenadine plasma concentrations in the control phase, the Ae_{0-24} of (*S*)-fexofenadine was slightly higher than that of (*R*)-fexofenadine (Fig. 9). Although the mean CL_{renal} of (*S*)-fexofenadine was significantly higher than that of (*R*)-fexofenadine ($P < 0.01$), the mean Ae_{0-24} values were not different between the (*R*)- and (*S*)-enantiomers. During the rifampicin pretreatment phase, the Ae_{0-24} of (*S*)-fexofenadine was not different between the control and rifampicin phases, even though rifampicin significantly increased the plasma concentrations of both fexofenadine enantiomers (Fig. 9). While the Ae_{0-24} of (*R*)-fexofenadine was markedly decreased in the rifampicin phases ($P < 0.05$) (Fig. 9 and Table 4), and then there were significant differences in the mean Ae_{0-24} values between the (*R*)- and (*S*)-fexofenadine enantiomers ($P < 0.001$) (Table 4). Although rifampicin significantly decreased the CL_{renal} of both enantiomers ($P < 0.01$ for both enantiomers), the mean individual differences for the CL_{renal} of (*R*)-fexofenadine had a greater trend compared with those of (*S*)-fexofenadine ($P < 0.001$) (Fig. 10B). From the above-mentioned results, the mean CL_{renal} *R/S* ratio of 0.64 (95% CI, 0.58-0.69) was slightly decreased to 0.59 (95% CI, 0.52-0.66) in the rifampicin phase; however, the mean CL_{renal} *R/S* ratio was not different between control and rifampicin phase (Table 4).

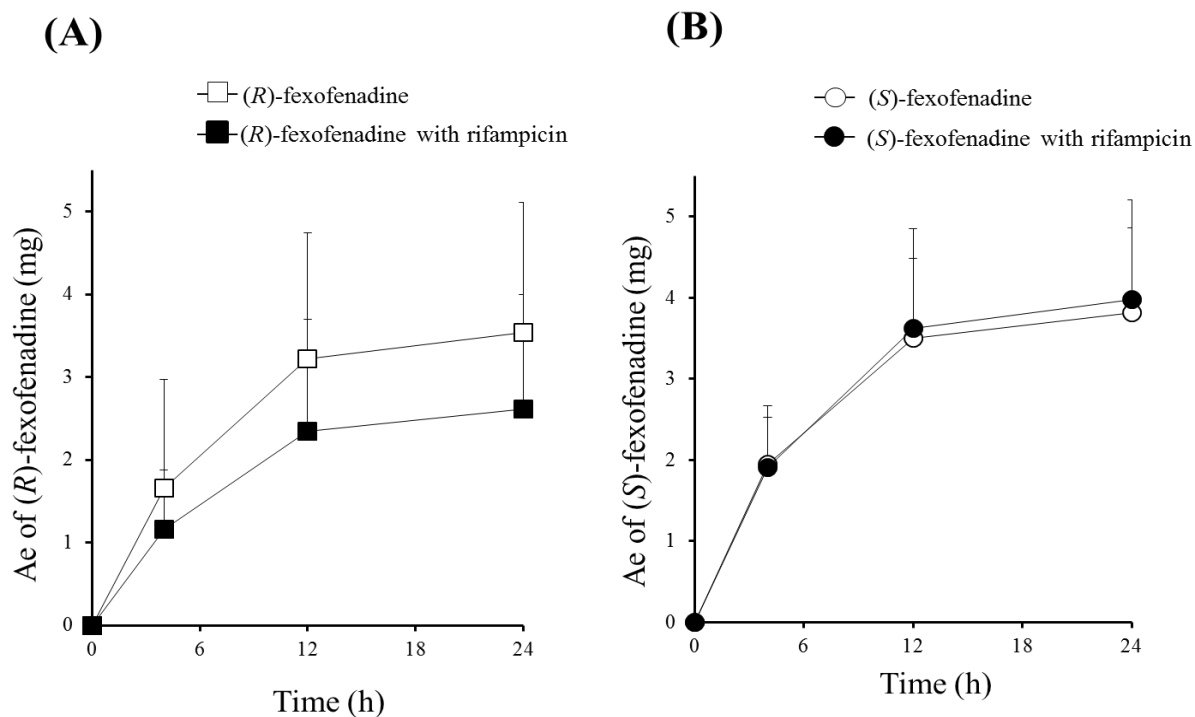


Fig. 9.

(A) Mean (+ SD) cumulative amount of (R)-fexofenadine excreted into urine following a single oral administration of 60 mg fexofenadine hydrochloride in ten healthy volunteers treated with placebo (open squares) or rifampicin (closed squares). (B) Mean (+ SD) cumulative amount of (S)-fexofenadine excreted into urine following a single oral administration of 60mg fexofenadine hydrochloride in ten healthy volunteers treated with placebo (open circles) or rifampicin (closed circles).

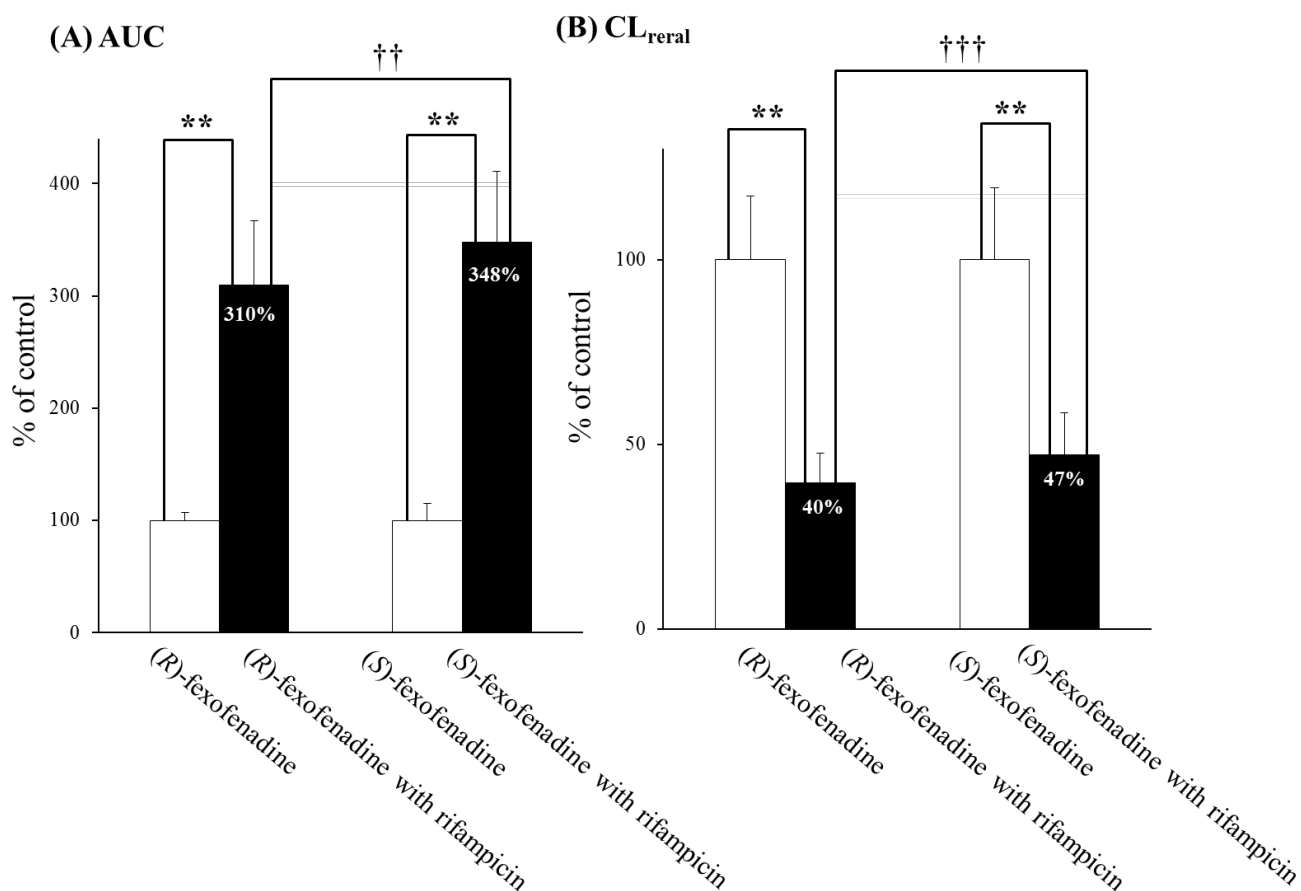


Fig. 10.

(A) The differences between the control (open bars) and rifampicin-treated groups (closed bars) for the mean AUC_{0-24} of (R)- and (S)-fexofenadine. (B) The differences between the control (open bars) and rifampicin-treated groups (closed bars) for the mean CL_{renal} of (R)- and (S)-fexofenadine.

Data are shown as the mean + SEM.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, between control phase and rifampicin phase.

† $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$, between (R)- and (S)-fexofenadine.

Table 4.

Effect of rifampicin on pharmacokinetic parameters of fexofenadine enantiomers

Parameters	With control	With rifampicin	Ratio to control
(R)- fexofenadine			
$t_{1/2}$ (h)	4.1 (3.3-5.0)	3.5 (2.9-4.0) ^{††}	0.87 (0.73-1.25)
t_{max} (h)(range)	1.4 (0.5-3.0)	1.8 (1.0-4.0)	1.70 (0.90-2.50)
C_{max} (ng/mL)	126 (100-152) ^{†††}	364 (300-428) ^{***†}	3.08 (2.51-3.65)
AUC_{0-24} (ng·h/mL)	739 (638-840) ^{††}	2205 (1386-3023) ^{***††}	3.10 (1.98-4.22)
CL/F (L/h)	43 (36-49) ^{†††}	17 (12-22) ^{***†††}	0.43 (0.27-0.59)
Ae_{0-24} (mg)	3.5 (2.5-4.4)	2.5 (1.9-3.0) ^{*†††}	0.83 (0.56-1.08)
CL_{renal} (L/h)	4.5 (3.0-6.0) ^{††}	1.4 (1.0-1.7) ^{***†††}	0.40 (0.24-0.55)
(S)-fexofenadine			
$t_{1/2}$ (h)	3.4 (2.3-4.5)	2.8 (2.5-3.0)	0.82 (0.56-1.09)
t_{max} (h)(range)	1.6 (1.0-4.0)	1.8 (1.0-4.0)	1.64 (0.83-2.45)
C_{max} (ng/mL)	104 (83-125)	326 (266-387) ^{***}	3.35 (2.56-4.13)
AUC_{0-24} (ng·h/mL)	522 (366-677)	1563 (1069-2057) ^{**}	3.48 (2.25-4.70)
CL/F (L/h)	67 (52-82)	23 (17-30) ^{***}	0.42 (0.22-0.61)
Ae_{0-24} (mg)	3.8 (2.8-4.7)	3.7 (2.8-4.5)	1.12 (0.77-1.48)
CL_{renal} (L/h)	7.3 (4.5-10.1)	2.4 (1.6-3.1) ^{**}	0.47 (0.25-0.69)
R/S ratio of AUC_{0-24}	1.54 (1.38-1.73)	1.39 (1.30-1.48)	0.94 (0.78-1.11)
R/S ratio of Ae_{0-24}	0.92 (0.89-0.94)	0.68 (0.64-0.72) ^{***}	0.74 (0.69-0.80)
R/S ratio of CL_{renal}	0.64 (0.58-0.69)	0.59 (0.52-0.66)	0.95 (0.80-1.11)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, between control phase and rifampicin phase.† $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$, between (R)- and (S)-fexofenadine.Data are shown as mean and 95% confidence interval; t_{max} data are shown as a median with a range.

3.1.4. Discussion

We evaluated the effects of multiple 450 mg doses of rifampicin on fexofenadine enantiomers pharmacokinetics in Japanese healthy volunteers. Similar to the results of previous 600 mg doses study [43], rifampicin significantly increased the mean AUC_{0-24} and C_{max} of both enantiomers, although, in the control phase, there were significant differences between the plasma concentrations of (*R*)- and (*S*)-fexofenadine (Fig. 8 and Table 4). Therefore, these results suggest that multiple 450 mg doses rifampicin probably inhibited the OATPs-mediated transport of both enantiomers, and this effect may be greater than the induction effect on P-gp. A previous *in vitro* study suggested that OATP subtypes 1B1 and 1B3 mainly contribute to the hepatic uptake of fexofenadine [65]. Since our previous report suggested that rifampicin inhibited the uptake of fexofenadine enantiomers by human hepatocytes via OATP1B3 [43], the present drug interactions also show that rifampicin may have inhibited fexofenadine at the liver uptake site through OATP1B3-mediated transport. These results therefore suggest that OATP1B3 is key determinant on fexofenadine enantiomers pharmacokinetics.

However, our previous report showed that multiple 600 mg doses rifampicin pretreatment significantly increased the mean $AUC_{0-\infty}$ of (*R*)- and (*S*)-fexofenadine (2.40-fold and 3.13-fold, respectively) [43], whereas the present multiple 450 mg doses study increased the mean AUC_{0-24} by 3.10-fold for (*R*)-fexofenadine and by 3.48-fold for (*S*)-fexofenadine (Fig. 10A). In the present study, notably the magnitude of the interaction on the mean AUC values of both enantiomers was higher compared with previous 600 mg doses study and then there was not observed dose-dependent inhibitory effect of rifampicin. Therefore, since it is potential that rifampicin is given

concomitantly with fexofenadine in the clinical situations, these findings show that sufficient monitoring will be required for patients receiving fexofenadine because of the increase in its plasma concentrations by rifampicin.

Previous *in vitro* study showed that rifampicin inhibits OATP1B3-mediated uptake of fexofenadine enantiomers, and the specific uptake by OATP1B3 was abolished at 2 μM [43]. Since rifampicin concentrations of 450 mg multiple doses are higher than 2 μM [81], this finding therefore may imply that OATPs-inhibited effects of rifampicin may be saturated by multiple 450 mg doses of clinical situation. Moreover, results of two different doses studies might be indicated that the induction of P-gp by 600 mg doses of rifampicin may be greater extent than that of 450 mg doses rifampicin, resulting in the AUC increase of 450 mg study was greater than that of 600 mg study. This is, the interactive mechanism of rifampicin multiple doses may be occurred though the combination of OATPs and P-gp transporters, which results in a somewhat change of fexofenadine enantiomers pharmacokinetics.

Moreover, rifampicin strongly elevated the mean AUC_{0-24} values of both enantiomers, the mean individual differences between the control and rifampicin phases for the AUC_{0-24} of (*S*)-fexofenadine were greater than those of (*R*)-fexofenadine (Fig. 10A). Although rifampicin decreased the mean AUC_{0-24} *R/S* ratio from 1.54 to 1.39, but this difference was not significant (Table 4). This result implies that OATP1B3 does not play an important role in the stereoselective pharmacokinetics of fexofenadine. The first chapter indicated that the pharmacokinetics of (*S*)-fexofenadine are affected by a polymorphism of *SLCO2B1*. Additionally, a previous clinical study reported that grapefruit juice decreases the oral bioavailability of fexofenadine, and this interaction may be caused by the inhibition of intestinal OATPs, such as 1A2 and 2B1 [51].

However, our present study was not able to confirm whether what extent OATP2B1 and 1A2 contributed to the stereoselectivity of fexofenadine because the inhibitory effect of rifampicin was a lesser extent for these intestinal uptake transporters.

Contrary to the observed plasma concentrations, we demonstrated that the Ae_{0-24} of (*S*)-fexofenadine was slightly higher than that of (*R*)-fexofenadine, and the mean CL_{renal} of (*S*)-fexofenadine was significantly greater during the control phase (Fig. 9 and Table 4). Rifampicin significantly decreased the mean CL_{renal} of both enantiomers, and this effect was greater for (*R*)-fexofenadine than for (*S*)-fexofenadine (Fig. 10B). Consequently, rifampicin decreased the mean *R/S* ratio of Ae_{0-24} from 0.92 to 0.68 because the Ae_{0-24} of (*R*)-fexofenadine decreased to a greater extent (Table 4). As for these findings, by now, although there are not suitable *in vitro* and *in vivo* evidences, they do support one hypothesis. In a previous study, we indicated that the organic anion transporters (OATs) inhibitor, probenecid, decreased the renal clearance of racemic fexofenadine in healthy subjects [48]. Because fexofenadine is a substrate of OAT3 but not OAT1 and OAT2 [82], this drug interaction mechanism can probably be explained by the inhibition of OAT3-mediated renal uptake by rifampicin. Therefore, there is a potential that the inhibitory effect of OAT3 by rifampicin is greater for (*R*)-fexofenadine. However, although OAT3-transfected cells showed significantly greater uptake of fexofenadine enantiomers, which were not inhibited by rifampicin [43]. In addition, significant uptake of both enantiomers were observed in MATE1-transfected cells, and this effect was slightly higher for (*R*)-fexofenadine [43]. But rifampicin also did not show inhibitory effect on MATE1 [43]. Therefore, tubular secretion may involve other unknown transporters that are sensitive to rifampicin, we are currently conducting *in vitro* studies to elucidate this potential mechanism.

3.1.5. Conclusion

In conclusion, this study suggests that multiple 450 mg doses of rifampicin may be sufficient to inhibit the OATPs-mediated hepatic uptake of both enantiomers and probably inhibit the renal influx transporter, and could possibly cause a clinical significance for patients receiving fexofenadine. Meanwhile, these effects may be greater compared to the P-gp-inductive effects by rifampicin. Therefore, this interactive mechanism of rifampicin multiple doses may be occurred though the combination of OATPs and P-gp transporters, which results in a somewhat change of fexofenadine enantiomers pharmacokinetics.

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

3.2.1. Introduction

Fruit juices are known to cause drug interactions with OATP substrate drugs [60]. 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 [18-23,51]. OATP1A2 and OATP2B1 are expressed on the luminal membrane of small intestinal enterocytes, potentially participating in the active absorption of drugs [2]. Both juices have been found to inhibit OATP1A2 and OATP2B1 *in vitro* [83-85]; therefore, currently, inhibition of the OATPs-mediated intestinal uptake is considered as the mechanism underlying a significant reduction in the oral bioavailability of the substrate drugs. In addition to grapefruit and orange juices, apple juice also significantly decreases the bioavailability of drugs, such as aliskiren and fexofenadine [18,51]. Although apple juice had no effect on OATP1A2 activity in an *in vitro* study [51], the latest study showed that apple juice strongly inhibits OATP2B1 activity *in vitro* [86-88]. Apple juice is more potent OATP2B1 inhibitor than grapefruit and orange juices *in vitro* [88]. Therefore, it is considered that the primary mechanism of the interactions with apple juice is also involves the inhibition of OATP2B1-influx transport in the small intestine.

Fexofenadine's pharmacokinetics primarily depends on the activities of multiple transporters; including P-gp, OATPs and MATE1 [40,52-54]. In the second chapter and previous content (3.1.), these studies demonstrated that carbamazepine and

rifampicin affected stereoselectivity in fexofenadine pharmacokinetics. However, the latest study showed that the activity of both fexofenadine enantiomers was identical in the P-gp, OATP1B3, OAT3 or MATE1-expressing cells [43]. Therefore, it is possible that other transporters may also contribute to the stereoselective pharmacokinetics of fexofenadine.

Meanwhile, first chapter reported that *SLCO2B1* (encoding OATP2B1) polymorphisms are associated with the pharmacokinetics of fexofenadine enantiomers. It remains unclear to what extent OATP2B1-mediated transport contributes to the stereoselectivity of fexofenadine. When the stereoselective disposition of fexofenadine is also caused by OATP2B1-mediated transport, apple juice may influence stereoselective pharmacokinetics of fexofenadine enantiomers. In addition, although racemic fexofenadine pharmacokinetics was reduced the bioavailability by repeated high volume (1200 mL) ingestions of apple juice [51], there is no information on the effect by one-time ingestion of apple juice. Therefore, we examined whether one-time single apple juice ingestion affects fexofenadine enantiomers pharmacokinetics and whether apple juice alters the stereoselectivity of fexofenadine. Subsequently, we conducted *in vitro* studies by OATP2B1 cRNA-injected oocytes to support the clinical data.

3.2.2. Subjects and study design

Fourteen healthy Japanese volunteers (ten males and four females) were enrolled in this study after providing informed written consent. 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 mean (\pm SD) age and body weight of the volunteers were 24.9 (\pm 5.7) years (range 20-42 years) and 57.5 (\pm 8.3) kg (range 44-70 kg), respectively. This study was approved by the Ethics Committee of the Hirosaki University School of Medicine, and all subjects gave their written informed consent before participating.

A randomized crossover study design in two phases was conducted at intervals of at least 2 weeks (Fig. 11). 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.. Apple juice (Shiny Apple[®]; Aomoriken Ringo Juice Co. Ltd., Aomori, Japan) used in this study was of normal strength. Volunteers did not take 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 hours after fexofenadine administration. In addition, the use of alcohol, tea and coffee was forbidden during the test period.

3.2.3. Results

Effect of apple juice on the plasma concentrations 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 fexofenadine hydrochloride are shown in Fig. 12 for both the control (with water) and apple juice-treated phases, and the pharmacokinetic parameters are summarized in Table 5.

One-time ingestion of apple juice greatly reduced the plasma concentration of both fexofenadine enantiomers compared with those of the water phase (Fig. 12) and altered all pharmacokinetic parameters except for the $t_{1/2}$ (Table 5). On the other hand, in the water phase, the plasma concentration of (*R*)-fexofenadine at all time points were higher than that of the corresponding (*S*)-enantiomer, and the mean AUC_{0-24} *R/S* ratio was 1.51 (95% CI, 1.40-1.63) (Fig. 12 and Table 5). Apple juice markedly decreased the AUC_{0-24} values of both enantiomers in all subjects ($P < 0.001$ for 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, respectively) (Fig. 13). Moreover, although there was no significant difference in the mean $t_{1/2}$ of the enantiomers in the control and apple juice-treated phase, the mean t_{max} of both enantiomers was markedly prolonged by apple juice ($P < 0.001$ for both enantiomers) (Table 5).

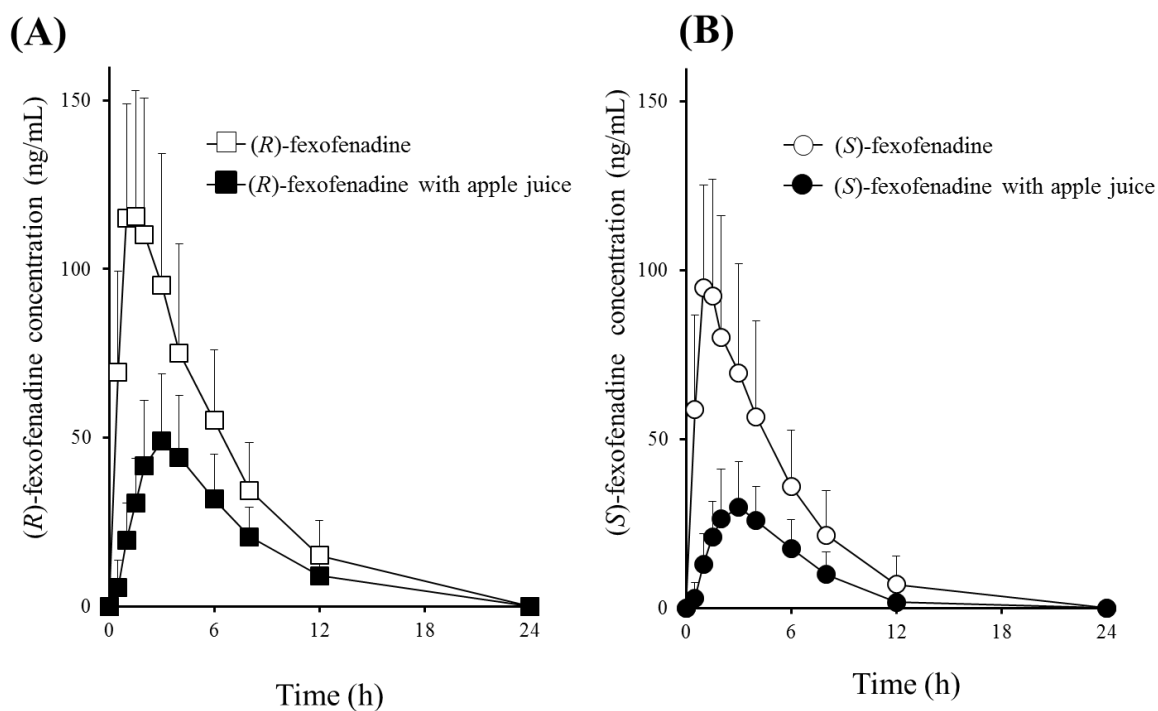


Fig. 12.

(A) Mean (+SD) plasma concentration-time curves of (R)-fexofenadine following a single oral administration of 60 mg fexofenadine hydrochloride in fourteen 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 fexofenadine hydrochloride in fourteen healthy volunteers treated with water (open circles) or apple juice (closed circles).

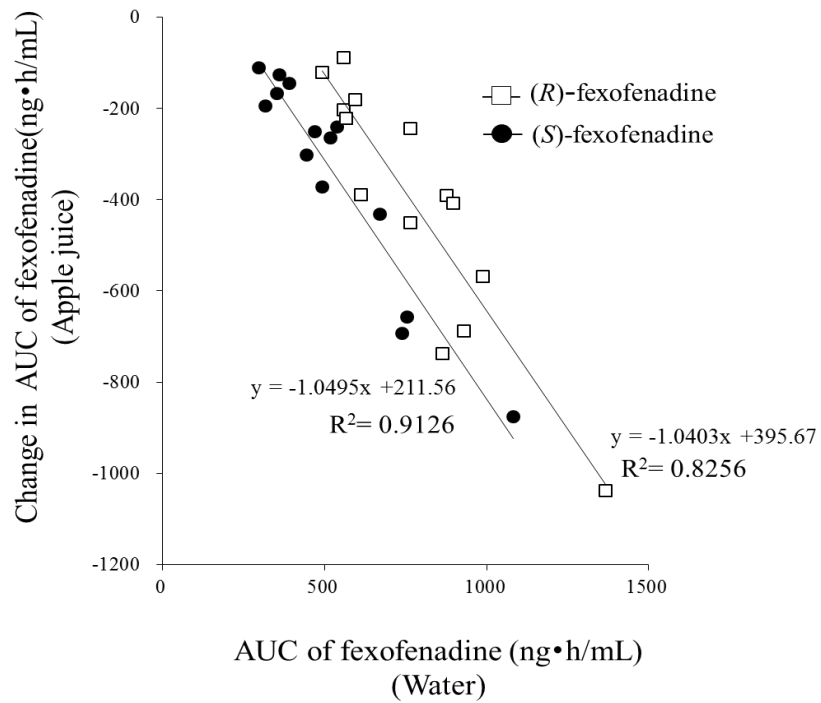


Fig. 13.

Change in both enantiomers 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).

Effect of apple juice on the urinary excretion of fexofenadine enantiomers

The mean values (+ SD) for the Ae_{0-24} of the fexofenadine enantiomers in both phases are shown in Fig. 14. In the control phase, the Ae_{0-24} of (*S*)-fexofenadine was slightly higher than that of (*R*)-fexofenadine (Fig. 14), however, the difference did not reach statistical significance ($P = 0.541$) (Table 5). The mean CL_{renal} of (*S*)-fexofenadine was significantly higher than that of (*R*)-fexofenadine ($P < 0.001$) (Table 5). 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 (*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 decreased the mean *R/S* ratio of CL_{renal} from 0.60 (95% CI, 0.55-0.65) to 0.49 (95% CI, 0.42-0.55), but this difference was not significant ($P = 0.081$) (Table 5).

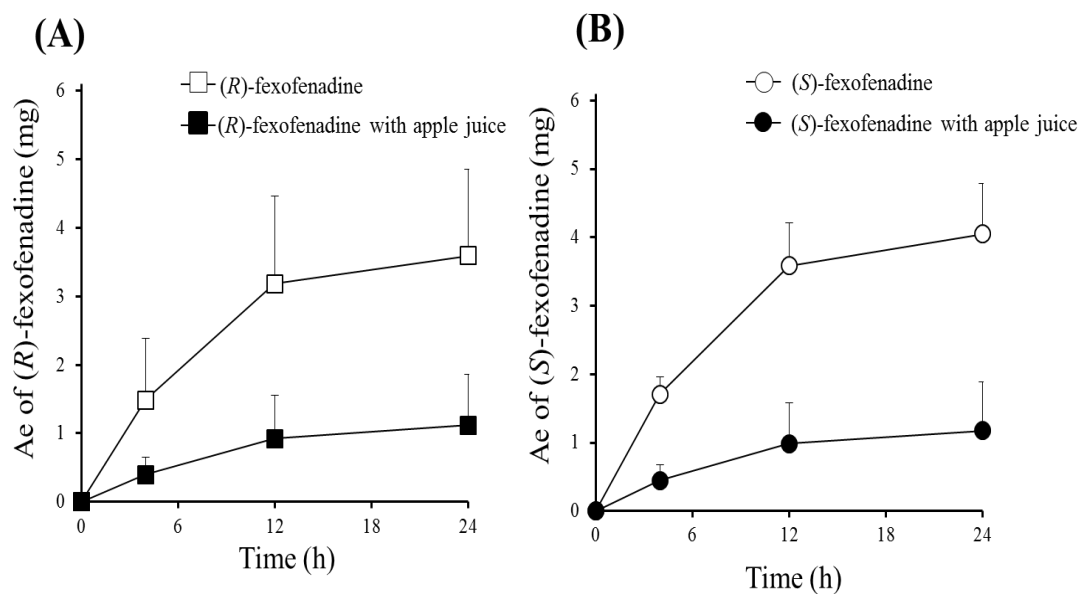


Fig. 14.

(A) Mean (+ SD) cumulative amount of (*R*)-fexofenadine excreted into urine following a single oral administration of 60 mg fexofenadine hydrochloride in fourteen healthy volunteers during the water (open squares) or apple juice (closed squares). (B) Mean (+ SD) cumulative amount of (*S*)-fexofenadine excreted into urine following a single oral administration of 60mg fexofenadine hydrochloride in fourteen healthy volunteers during the water (open circles) or apple juice (closed circles).

Table 5.

Effect of apple juice on pharmacokinetic parameters of fexofenadine enantiomers

Parameters	Control	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·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·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)
R/S ratio of AUC_{0-24}	1.51 (1.40-1.63)	2.07 (1.87-2.28) **	1.41 (1.19-1.64)
R/S ratio of CL_{renal}	0.60 (0.55-0.65)	0.49 (0.42-0.55)	0.84 (0.70-0.99)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, between control phase and apple juice phase.† $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$, 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.

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

To determine whether fexofenadine enantiomers are transported by OATP2B1, uptake of both enantiomers were measured using *Xenopus* oocytes expressing OATP2B1 (Fig. 15). The uptake of (*R*)- and (*S*)-fexofenadine (100 μ M) by OATP2B1 cRNA-injected oocytes were significantly higher than that by water-injected oocytes, and this uptake was 2.2 times greater for (*R*)-fexofenadine ($P < 0.001$) (Fig. 15). In addition, OATP2B1-mediated uptake of both enantiomers were significantly decreased in the presence of 10 % apple juice ($P < 0.001$ for both enantiomers) (Fig. 15).

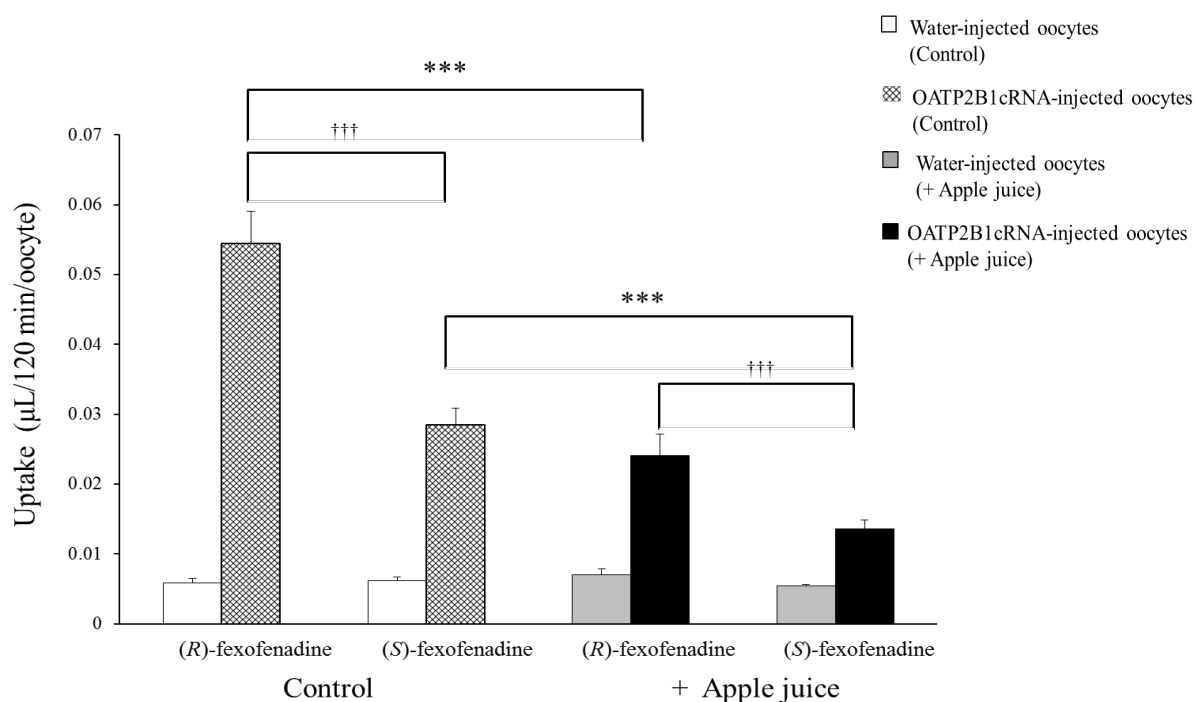


Fig. 15. Uptake of (R)- and (S)-fexofenadine by *Xenopus* oocytes expressing OATP2B1.

Uptake of (R)-, (S)-fexofenadine (100 µM) by 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) were measured in the presence or absence of 10% AJ for 120 min at 25°C and pH 6.5. Data are shown as the mean + SEM (n = 4-9).

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, between control phase and apple juice phase.

† $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$, between (R)- and (S)-fexofenadine.

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

Inhibitory effects of apple juice on (R)- and (S)-fexofenadine were represented as percentage of control (without apple juice) in Fig. 16. In the *human study*, apple juice markedly decreased the AUC₀₋₂₄ for (R)- and (S)-fexofenadine by 49% and 59%, respectively ($P < 0.001$ for both enantiomers), and these inhibited-effects were significant differences between (R)- and (S)-fexofenadine ($P < 0.001$) (Fig. 16A). Therefore, apple juice increased the mean *R/S* ratio of the AUC₀₋₂₄ from 1.51 (95% CI, 1.40-1.63) to 2.07 (95% CI, 1.87-2.28) ($P < 0.01$) (Table 5). Meanwhile, in the *in vitro study*, apple juice significantly decreased OATP2B1-mediated uptake for (R)- and (S)-fexofenadine by 65% and 64%, respectively ($P < 0.001$ for both enantiomers), but these inhibited-effects were identical between both enantiomers ($P = 0.888$) (Fig. 16B).

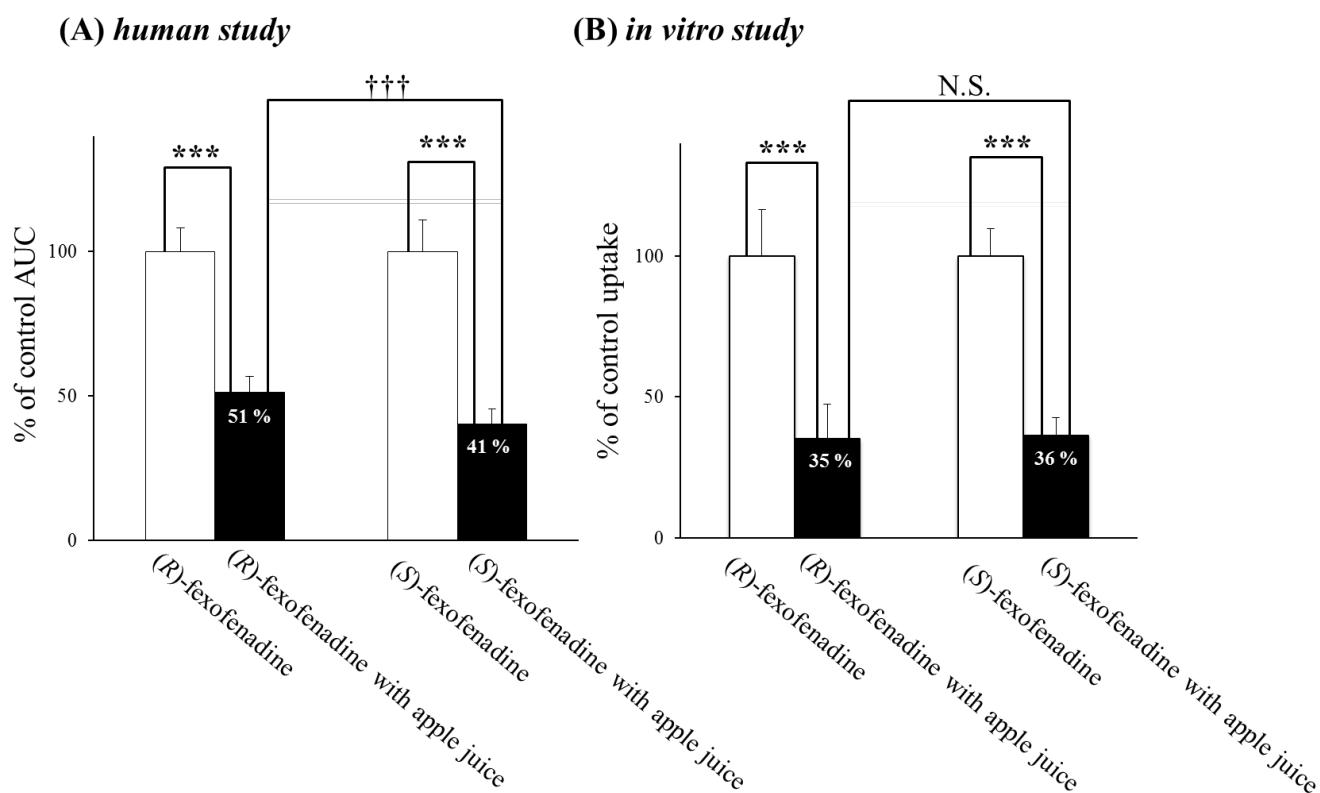


Fig. 16. Inhibitory effect of apple juice on (R)- and (S)-fexofenadine in *in vivo* and *in vitro* study

(A) The differences between the control (open bars) and apple juice-treated groups (closed bars) for the mean AUC_{0-24} of (R)- and (S)-fexofenadine following a single oral administration of 60 mg fexofenadine hydrochloride in fourteen healthy volunteers. (B) The differences between the control (open bars) and apple juice-treated groups (closed bars) for the mean OATP2B1-mediated uptake of (R)- and (S)-fexofenadine (100 μ M) by OATP2B1-expressing *Xenopus* oocytes (n = 6-9).

Data are shown as the mean + SEM.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, between control phase and apple juice phase.

† $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$, between (R)- and (S)-fexofenadine.

N.S., Not Significant.

3.2.4. Discussion

The authors investigated the effects of one-time apple juice ingestion on the pharmacokinetics of fexofenadine enantiomers. This study also showed that apple juice significantly decreased the mean C_{\max} and AUC_{0-24} of both enantiomers even by a single ingestion (Fig. 12 and Table 5). When this effect of apple juice is attributable to an inhibition of hepatic OATP-influx transport, the plasma concentrations of both enantiomers would have been rather enhanced as described previously [12-16]. Therefore, apple juice should inhibit intestinal absorption via OATPs. This is also supported by the fact that the mean t_{\max} of both enantiomers was markedly prolonged by apple juice (Table 5). It is possible that inhibition of OATPs-influx transport by apple juice leads to a decrease in the distribution volume of both enantiomers and a simultaneous delay in their absorption.

Previous *in vitro* and *in vivo* studies found that the uptake of fexofenadine was significantly increased in OATP2B1 cRNA-injected oocytes, and that this uptake was decreased in the presence of apple juice [86]. Similarly, in the present study [86], the uptake of both fexofenadine enantiomers by OATP2B1 cRNA-injected oocytes was significantly higher than that by water-injected oocytes (Fig. 15), and apple juice significantly decreased the uptake of both enantiomers by OATP2B1 cRNA-injected oocytes (Fig. 15 and Fig. 16). Therefore, these results suggest that intestinal OATP2B1 plays an important role on the pharmacokinetics of fexofenadine enantiomers, and apple juice may have inhibited OATP2B1-mediated intestinal transport.

Additionally, a previous clinical study showed that repeated ingestions of apple juice reduces the AUC of racemic fexofenadine by approximately 70 % of the

control [51], while our single-intake study showed that the AUC_{0-24} of (*R*)- and (*S*)-fexofenadine was decreased by 49 % and 59 %, respectively (Fig. 16). These findings probably indicate that the inhibitory effect of apple juice may increase with repeated ingestions of high volumes. However our present study suggests that one-time ingestion apple juice might be sufficient for the intestinal OATP2B1-inhibited effect, and could possibly cause a moderately clinical significance for patients receiving OATP2B1 substrate drugs such as fexofenadine.

Moreover, the inhibited-effects of apple juice on both fexofenadine enantiomers were dependent on the control AUC_{0-24} value of each enantiomer, and these showed the high correlations because apple juice caused a greater reduction in subjects with a higher control AUC_{0-24} values (Fig. 13). This result consistent with previous report [51], and implies that the higher baseline AUC_{0-24} values observed in the control phase may have been due to higher intestinal OATP2B1 activity. A previous *in vivo* study indicate that *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 of carrying *3 (c. 1457C>T) allele subjects [86]. Therefore, it is possible that the individual pharmacokinetics of fexofenadine enantiomers may be affected by OATP2B1 activity, and the different reductions of OATP2B1 activity by apple juice may be related to the individual apple juice-effects on the fexofenadine stereoselective pharmacokinetics.

Consistent with previous clinical reports [56,57,59,60], the present study demonstrated that the plasma concentration of (*R*)-fexofenadine was higher than the corresponding (*S*)-enantiomer during the control phase (Fig. 12). Interestingly, the uptake by OATP2B1 cRNA-injected oocytes was 2.2 times higher for (*R*)-fexofenadine

(Fig. 15). This result indicates that OATP2B1-mediated transport is stereoselectivity, and the higher absorption of (*R*)-fexofenadine may have been due to the high affinity of OATP2B1 for (*R*)-enantiomer. These results therefore suggest that OATP2B1 is a key determinant on the stereoselective pharmacokinetics of fexofenadine. In addition, in our previous *in vitro* study, although the stereoselectivity was not observed in the transport of fexofenadine enantiomers by P-gp, OATP1B3, OAT3 and MATE1-expressing cells [43], the plasma unbound fraction of (*S*)-fexofenadine showed 1.8 times higher than that of (*R*)-fexofenadine and it might be contributed to the fexofenadine stereoselective pharmacokinetics [43]. Taken together, these findings suggest that the stereoselectivity of fexofenadine may be occurred though the mixed combination of OATP2B1 activity and the plasma unbound fractions for each enantiomer, resulting in the higher plasma concentration of (*R*)-fexofenadine enantiomer.

3.2.5. Conclusion

In conclusion, these results suggest that OATP2B1 plays an important role on the stereoselective pharmacokinetics of fexofenadine, and one-time apple juice ingestion probably inhibited the intestinal OATP2B1-mediated transport of both enantiomers. Therefore, this study indicates that the OATP2B1-inhibited effects will not need repeated injections or high volume of apple juice.

General Conclusion

This manuscript focused on the drug-transporters contributing to the stereoselective pharmacokinetics using fexofenadine because it is poorly metabolized by CYPs. In the first chapter, we investigated the association of *SLCO* (encoding OATP, *1B1*, *1B3* and *2B1*), *ABCB1* (P-gp), *ABCC2* (MRP2) and *ABCG2* (BCRP) polymorphisms with fexofenadine enantiomers pharmacokinetics. The pharmacokinetics of (*S*)-fexofenadine is associated with a single polymorphism of *SLCO2B1*, and combinations of several polymorphisms of *ABCB1* C1236T, C3435T and *ABCC2* C-24T. Meanwhile, there were no significant differences in the AUC₀₋₂₄ and the C_{max} for (*R*)-fexofenadine between the *SLCO*, *ABCB1*, *ABCC2* or *ABCG2* genotypes. Our findings suggest that the combination of plural transporters involving OATPs, P-gp and MRP2 react strongly to fexofenadine exposure in the small intestine and liver, resulting in different disposition between both enantiomers (Fig. 17).

Subsequently, based on the results of the first chapter, we investigated the transport activities using several transporter-inducers/inhibitors to confirm the extent of its contributions. In the second chapter, this study was to characterize the impact of the P-gp inducer carbamazepine on fexofenadine enantiomer pharmacokinetics. Carbamazepine significantly decreased the AUC₀₋₂₄ and the Ae₀₋₂₄ of (*R*)- and (*S*)-fexofenadine. The P-gp inducer showed a greater effect on pharmacokinetic parameters of (*S*)-fexofenadine. This study indicates that carbamazepine may alter the pharmacokinetics of fexofenadine enantiomers. However, since the inductive effect of carbamazepine to P-gp may be different

between the fexofenadine enantiomers can not eliminate, it is likely that other transporters, including OATP2B1 and MRP2, also contribute to the stereoselective pharmacokinetics of fexofenadine (Fig. 17).

In the third chapter, we examined the effect of rifampicin and apple juice on the pharmacokinetics of fexofenadine enantiomers. Multiple dose of rifampicin is a potent P-gp inducer, but several recent *in vitro/vivo* studies have shown that rifampicin is an OATPs inhibitor. Rifampicin pretreatment significantly increased the mean AUC_{0-24} of (*R*)- and (*S*)-fexofenadine. In addition, rifampicin significantly decreased the CL_{renal} of (*R*)- and (*S*)-fexofenadine, and marked differences in the mean Ae_{0-24} of both enantiomers in the rifampicin phase were observed. These results indicate that multiple 450 mg doses of rifampicin may be sufficient to inhibit the OATPs-mediated hepatic uptake of both enantiomers and probably inhibit the renal influx transporter, and could possibly cause a clinical significance for patients receiving fexofenadine (Fig. 17). In addition, apple juice is also potent OATPs inhibitor, probably via OATP2B1. One-time ingestion of apple juice significantly decreased the AUC_{0-24} for (*R*)- and (*S*)-fexofenadine, and prolonged the t_{max} of both enantiomers. Although apple juice greatly reduced the Ae_{0-24} for (*R*)- and (*S*)-fexofenadine, the CL_{renal} of both enantiomers were not changed between control and apple juice phases. The uptake of both fexofenadine enantiomers by OATP2B1 cRNA-injected oocytes was significantly higher than that by water-injected oocytes, and these effects were greater for (*R*)-fexofenadine. Moreover, apple juice significantly decreased the uptake of both enantiomers by OATP2B1 cRNA-injected oocytes. These results suggest that OATP2B1 plays an important role on the stereoselective pharmacokinetics of fexofenadine, and one-time apple juice ingestion probably inhibited the intestinal

OATP2B1-mediated transport of both enantiomers (Fig. 17).

Taken together, our findings indicate that drug-transporters might have the ability of chiral discrimination. Since there is no report that drug transporters would contribute to the stereoselectivity in the *in vivo* and *in vitro*, we believe this study results are novel and will help clinical pharmaceutical research.

Meanwhile, until now, differences in the pharmacological effects and safety profiles for the fexofenadine enantiomers have not been established. Although Robbins *et al.* reported that the two enantiomers are pharmacologically identical [55], our recent *in vitro* binding assays demonstrated that (*S*)-fexofenadine is a more potent human histamine H1 receptor antagonist than (*R*)-fexofenadine [43]. Therefore, (*S*)-fexofenadine shows larger receptor occupancy, and consequently makes a greater contribution to the pharmacological response than (*R*)-fexofenadine. Although the clinical relevance of enantiomer effects is not yet fully established, the plasma concentrations of (*R*)-fexofenadine were consistently higher than those of the (*S*)-enantiomer. In addition, (*S*)-fexofenadine were more influenced by P-gp and OATPs inducer/inhibitors as compared to those of the corresponding (*R*)-enantiomers. Thus, the use of only (*R*)-fexofenadine might be more predictable therapeutic effect and less drug–drug interactions. It is thought that a further study is necessary in the future.

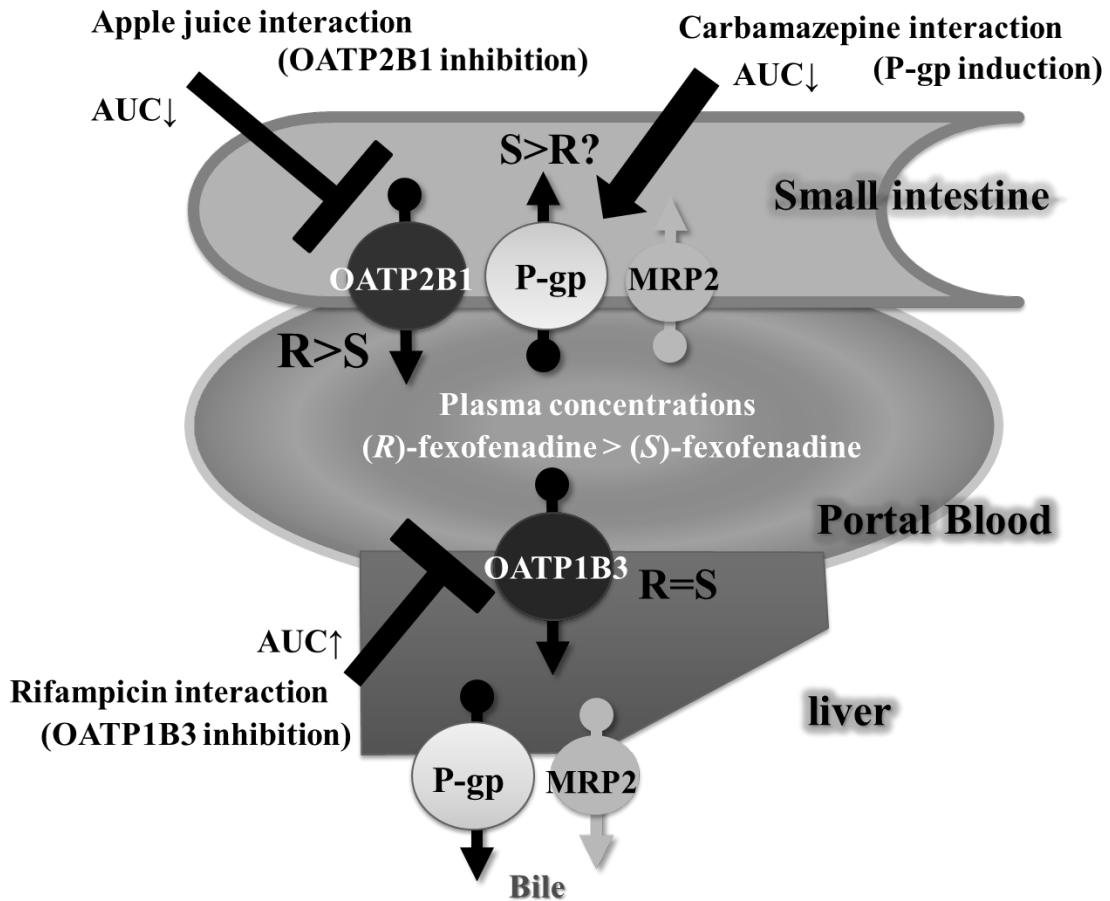


Fig. 17. Predictive mechanism.

AUC = area under the plasma concentration-time curve; MRP = multidrug resistance-associated protein; OATP = organic anion-transporting polypeptide; P-gp = P-glycoprotein.

Material and Methods

Chemicals and reagents (Chapter 1,2,3)

Fexofenadine and diphenhydramine were donated by Sanofi Aventis (Tokyo, Japan) and Tanabe Pharmaceutical Company Ltd. (Osaka, Japan), respectively. Fexofenadine enantiomers were fractionated by high pressure liquid chromatography (HPLC) using a chiral column, and then the elution order of the enantiomers in the HPLC chromatogram was determined from the reported (*R*)- and (*S*)-rotations [55]. An Oasis HLB extraction cartridge was purchased from Waters (Milford, MA, USA). All other reagents were purchased from Nacalai Tesque (Kyoto, Japan). All solvents were of HPLC grade.

Determination of fexofenadine enantiomer concentrations (Chapter 1,2,3)

Blood samples (10 mL each) were drawn into heparinized tubes before and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours after the administration of fexofenadine; the plasma was immediately separated. Just before fexofenadine administration, urine was collected for a blank sample. After fexofenadine administration, urine samples were collected for the following 24 hours. The plasma and urine samples were stored at – 20 °C until they were assayed.

The fexofenadine concentrations in plasma and urine were determined using an HPLC method that was developed in our laboratory [56]. Following the addition of diphenhydramine (50 ng) as an internal standard in methanol (10 µL) to 400 µL of plasma, the plasma sample was diluted with 600 µL of water and vortexed for 30 s. For the urine, a sample was diluted with 900 µL of water after diphenhydramine (50 ng) in methanol (10 µL) was added to a 100 µL urine sample. These mixtures were applied to an Oasis HLB extraction cartridge (Waters, Milford, MA, USA) that had been previously activated with methanol and water (1.0 mL each). The cartridge was then washed with 1.0 mL of water and 1.0 mL of 40 % methanol in water and eluted with 1.0 mL of 100 % methanol. Eluates were evaporated to dryness in a vacuum at 40 °C using a rotary evaporator (Iwaki, Tokyo, Japan). The residues were dissolved in 50 µL of methanol and vortexed for 30 s. Approximately 50 µL of mobile phase was added to each sample, and the samples were then vortexed for another 30 s. An aliquot of 50 µL of each sample was then processed using the HPLC apparatus. A Model 510 chromatography pump (Waters) equipped with a Waters 486 ultraviolet (UV) detector was used for the HPLC analysis. The HPLC column was a Chiral CD-Ph (250 mm x 4.6 mm i.d., Shiseido, Tokyo, Japan), and the mobile phase was 0.5 % KH₂PO₄ (pH

3.5)-acetonitrile (65:35, v:v) , which was degassed in an ultrasonic bath prior to use. Before mixing with acetonitrile, the pH of the 0.5 % KH_2PO_4 was adjusted with 50 % phosphoric acid. 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 (*R*)- and (*S*)-fexofenadine. The validated concentration ranges of this assay from plasma and urine were 25–625 ng/mL for both enantiomers. The within- and between-day coefficients of variation were less than 13.6 %, and accuracies were within 8.8 % over the linear range for both analytes. The plasma and urine blank samples that were collected before initiating fexofenadine administration had no detectable fexofenadine peak in the assay.

Genotype Identification (Chapter 1)

Single-nucleotide polymorphisms (SNPs) that influence enzyme activity were chosen. DNA was extracted from a peripheral blood sample using a QIAamp Blood Mini Kit (Qiagen, Tokyo, Japan) and was stored at -80 °C until analyzed. Genotyping procedures to identify *SLCO1B1* 1*a*, 1*b* and *15 alleles and *SLCO2B1* (C1467T) *1 and *3 alleles were performed using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method of Nozawa *et al.* [68]. Genotyping procedures identifying each allele of *SLCO1B3* (T334G) used the PCR-RFLP method described by Tsujimoto *et al.* [89]. Genotyping procedures identifying the C and T alleles in exon 1 of *ABCC2* (C-24T) and the C and T alleles in exon 12 (C1236T), the G and T/A alleles in exon 21 (C2677T), and the C and T alleles in exon 26 (C3435T) of *ABCB1* were performed by combining two PCR-RFLP methods described by Naesens *et al.* [90], Wu *et al.* [91], Tanaka *et al.* [92] and Cascorbi *et al.* [93], respectively. The *ABCG2* C421A polymorphism was genotyped by the PCR-RFLP method of Kobayashi *et al.* [94]. All frequencies for the different analyzed loci were at Hardy-Weinberg equilibrium.

Uptake Experiments in Xenopus laevis Oocytes (Chapter 3)

Preparation of oocytes, *in vitro* synthesis of OATP2B1 complementary RNA (cRNA), and uptake experiments were conducted as described previously [95]. The construct pGEMHE containing OATP2B1 complementary DNA was used to synthesize cRNA *in vitro*. Defolliculated oocytes were injected with 50 nL of the cRNA solution (1 $\mu\text{g}/\mu\text{L}$) or water, and then incubated for 3 days at 18°C in modified Barth's saline [MBS; 88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO₃, 0.82 mM MgSO₄, 0.33 mM Ca(NO₃)₂, 0.41 mM CaCl₂, and 10 mM HEPES, pH 7.4] containing 50 $\mu\text{g}/\text{mL}$ gentamicin (MBS). For the 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. The uptake was terminated by washing the oocytes three times with ice-cold MBS. The concentrations of fexofenadine in all 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-20AD ultra-fast LC system (Shimadzu Company, Kyoto, Japan). The ultra fast 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 for 0-1.25 min, 5.0-95 % acetonitrile for 1.25-2.25 min, 95 % acetonitrile for 2.25-4.35 min, 95-5.0 % acetonitrile for 4.35-4.5 min, and 5.0 % acetonitrile for 4.5-5.5 min. The total run time was 5.5 min for each injection. The retention times was 2.9 min. Mercury MS (C₁₈, 10×4.0 mm, Luna 3 mm, Phenomenex, Torrance, CA) was used as the analytical column. In the liquid chromatography-tandem mass spectrometry

system, the Turbo Ion Spray interface was operated in the positive ion mode at 5,500 V and at 700 °C. 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. Uptake rate ($\mu\text{L}/120 \text{ min}/\text{oocyte}$) was calculated as the cell-to-medium ratio by dividing the uptake amount by the initial concentration in the uptake medium. OATP2B1-mediated uptake rates were obtained after subtraction of the uptake by water-injected oocytes from that by OATP2B1 cRNA-injected oocytes.

Pharmacokinetic analysis (Chapter 1,2,3)

Pharmacokinetic analysis of fexofenadine enantiomers was done according to a standard non-compartmental method using WinNonlin software (Pharsight Co., Mountain View, CA, version 4.0.1). The C_{\max} and the t_{\max} of fexofenadine were determined directly from the observed data. The elimination rate constant (k_e) of fexofenadine was obtained by linear regression analysis using at least 3 sampling points of the terminal log-linear declining phase to the last measurable concentration. The $t_{1/2}$ was calculated as 0.693 divided by k_e . The AUC from time zero 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 $CL/F = \text{Dose}/AUC_{0-24}$, where Dose was 30 mg for each fexofenadine enantiomer. The CL_{renal} was obtained from the following equation: $CL_{\text{renal}} = Ae_{0-24}/AUC_{0-24}$, where Ae is the amount of fexofenadine excreted into the urine within a 24-hour period.

Statistical analysis (Chapter 1,2,3)

In the first chapter, all results are expressed as the median (range). Statistical significance of differences of kinetic parameters of each fexofenadine enantiomer between the two genotypes was evaluated with the Mann-Whitney *U* test using SPSS statistical software (SPSS Japan Inc., Tokyo, Japan, version 12.0). Statistical comparisons of the parameters were supplemented with the multiple comparison procedure by the Kruskal-Wallis test using SPSS statistical software. A *P*-value less than 0.05 was considered statistically significant.

In the second and third chapters, the results are expressed as mean and 95% CI in the table, and mean + standard deviation (SD) or mean + standard error of the mean (SEM) in the figures. Differences between (*R*)- and (*S*)-fexofenadine in their pharmacokinetic parameters during the control phase and apple-juice phase, in the *R/S* ratio of the AUC₀₋₂₄ during the control phase and the treatment phase, and in the mean difference (%) in the within-subject ratio 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 the 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.

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