Effects of organic anion transporting polypeptide 1B1 haplotype on pharmacokinetics of pravastatin, valsartan, and temocapril

Objective: **Recent reports have shown that genetic polymorphisms in organic anion transporting polypeptide (OATP) 1B1 have an effect on the pharmacokinetics of drugs. However, the impact of *OATP1B1*1b* alleles, the frequency of which is high in all ethnicities, on the pharmacokinetics of substrate drugs is not known after complete separation of subjects with *OATP1B1*1a* and **1b*. Furthermore, the correlation between the clearances of OATP1B1 substrate drugs in individuals has not been characterized. We investigated the effect of genetic polymorphism of OATP1B1, particularly the **1b* allele, on the pharmacokinetics of 3 anionic drugs, pravastatin, valsartan, and temocapril, in Japanese subjects.**

Methods: **Twenty-three healthy Japanese volunteers were enrolled in a 3-period crossover study. In each period, after a single oral administration of pravastatin, valsartan, or temocapril, plasma and urine were collected for up to 24 hours.**

Results: **The area under the plasma concentration–time curve (AUC) of pravastatin in **1b/*1b* carriers (47.4 ± 19.9 ng · h/mL) was 65% of that in **1a/*1a* carriers (73.2 ± 23.5 ng · h/mL) ($P = .049$). Carriers of **1b/*15* (38.2 ± 15.9 ng · h/mL) exhibited a 45% lower AUC than **1a/*15* carriers (69.2 ± 23.4 ng · h/mL) ($P = .024$). In the case of valsartan we observed a similar trend as with pravastatin, although the difference was not statistically significant (9.01 ± 3.33 μg · h/mL for **1b/*1b* carriers versus 12.3 ± 4.6 μg · h/mL for **1a/*1a* carriers [$P = .171$] and 6.31 ± 3.64 μg · h/mL for **1b/*15* carriers versus 9.40 ± 4.34 μg · h/mL for **1a/*15* carriers [$P = .213$]). The AUC of temocapril also showed a similar trend (12.4 ± 4.1 ng · h/mL for **1b/*1b* carriers versus 18.5 ± 7.7 ng · h/mL for **1a/*1a* carriers [$P = .061$] and 16.4 ± 5.0 ng · h/mL for **1b/*15* carriers versus 19.0 ± 4.1 ng · h/mL for **1a/*15* carriers [$P = .425$]), whereas that of temocaprilat (active form of temocapril) was not significantly affected by the haplotype of OATP1B1. Interestingly, the AUC of valsartan and temocapril in each subject was significantly correlated with that of pravastatin ($R = 0.630$ and 0.602, $P < .01$). The renal clearance remained unchanged for each haplotype for all drugs.**

Conclusion: **The major clearance mechanism of pravastatin, valsartan, and temocapril appears to be similar, and *OATP1B1*1b* is one of the determinant factors governing the interindividual variability in the pharmacokinetics of pravastatin and, possibly, valsartan and temocapril. (Clin Pharmacol Ther 2006;79:427-39.)**

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The administration of the same dose of a drug sometimes results in large interindividual differences in pharmacokinetics and subsequent pharmacologic and toxicologic effects. The pharmacokinetics of certain drugs are dominated by absorption, disposition, metabolism, and elimination, and many molecules, such as metabolic enzymes and transporters, have been reported to be involved in each process. Recently, polymorphisms in each molecule have been identified, and many in vitro and clinical studies have demonstrated that some of them are associated with a change in the expression and function of molecules and the pharmacokinetics of drugs. Although there is much information regarding metabolic enzymes such as cytochrome P450 (CYP) and phase II conjugation enzymes, the clinical significance of the genetic polymorphisms in transporters is not well understood.

Organic anion transporting polypeptide (OATP) 1B1 (formerly known as OATP-C or OATP2) is exclusively expressed in the liver and located on the basolateral membrane.[1–3] Some reports have indicated that OATP1B1 can transport a wide variety of compounds including clinically important drugs such as 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors,[1,4,5] which suggests that OATP1B1 may be responsible for the hepatic uptake of various kinds of anionic drugs, which efficiently accumulate in liver. Hepatic clearance consists of intrinsic clearances of hepatic uptake, sinusoidal efflux, metabolism, and biliary excretion. From the viewpoint of pharmacokinetics, a change in the uptake process will directly affect the overall hepatic clearance, regardless of the absolute values of each intrinsic clearance.[6] Therefore genetic polymorphisms in OATP1B1 may have an effect on the hepatic clearance of OATP1B1 substrates.

Several genetic polymorphisms in OATP1B1 have been reported, and in vitro studies have shown that some of them reduce the transport capability of several substrates in OATP1B1 variant–expressing cells.[7–9] Among these, previous studies have focused on 2 mutations, Asn130Asp and Val174Ala, because they are frequently observed in all ethnic groups investigated previously and their allele frequencies show some ethnic differences,[9,10] which may cause an ethnic difference in the pharmacokinetics of OATP1B1 substrates. Interestingly, Nishizato et al[10] demonstrated that Val174Ala was tightly linked with Asn130Asp and formed a haplotype referred to as *OATP1B1∗15* in Japanese subjects. In addition, after oral administration of pravastatin, healthy Japanese volunteers with the *∗15* allele showed an increase in the area under the plasma concentration–time curve (AUC) of pravastatin. This result was supported by in vitro analysis showing that the intrinsic maximum velocity normalized by the expression level for *OATP1B1∗15* variant was drastically reduced compared with *OATP1B1∗1a*.[7–9] Subsequently, 2 clinical studies showed that the Val174Ala mutation also increased the AUC of pravastatin in white subjects.[11,12] Very recently, Niemi et al[13,14] reported that the pharmacokinetics of fexofenadine and repaglinide was also affected by the Val174Ala mutation. These results suggest that the Val174Ala mutation in OATP1B1 reduces the transport function. On the other hand, Mwinyi et al[12] showed that the AUC of pravastatin in subjects with *∗1a/∗1b* (Asn130Asp) or *∗1b/∗1b* alleles tended to be lower than that in *∗1a* homozygotes. However, they did not completely separate the subjects with the *∗1b* allele from those with the *∗1a* allele, and so we cannot directly compare the effect of the *∗1b* allele with that of the *∗1a* allele. The allele frequency of *OATP1B1∗1b* was reported to be high and showed some ethnic differences (eg, 0.30 in white Americans [n = 49],[9] 0.74 in black Americans [n = 44],[9] and 0.63 in Japanese subjects [n = 120][10]), implying that this might cause the ethnic differences in the pharmacokinetics of drugs. Therefore we were particularly interested in the effect of the Asn130Asp variant of OATP1B1 on the pharmacokinetics of 3 drugs, pravastatin, valsartan, and temocapril, and we classified the subjects into 4 groups, *∗1a/∗1a*, *∗1b/∗1b*, *∗1a/∗15*, and *∗1b/∗15* carriers, to directly investigate the difference in the pharmacokinetics of the subjects with the *∗1a* and *∗1b* alleles (*∗1a/∗1a* versus *∗1b/∗1b* and *∗1a/∗15* versus *∗1b/∗15*).

Valsartan is a novel angiotensin II receptor antagonist, and temocapril is an angiotensin-converting enzyme inhibitor. Drugs in these categories are widely used for the treatment of hypertension. Valsartan is mainly eliminated via the liver. Valsartan itself is pharmacologically active and is thought to be excreted into the bile in unchanged form without extensive metabolism.[15] Because of its hydrophilicity and carboxyl moiety, some organic anion transporters may be involved in the hepatic clearance of valsartan. Temocapril is an esterified prodrug and is rapidly converted to the active metabolite temocaprilat by carboxyl esterase.[16] Temocaprilat is mainly excreted into the bile, whereas the active metabolites of other angiotensin-converting enzyme inhibitors such as enalaprilat are mainly excreted into the urine because temocaprilat, but not enalaprilat, can interact with multidrug resistance associated protein 2 (MRP2), which is an efflux transporter located on the apical membrane.[17] Sasaki et al[18] demonstrated that transcellular vectorial transport of temocaprilat was

observed in OATP1B1/MRP2 double-transfected cells, suggesting that temocaprilat is a substrate of OATP1B1.

Therefore the purpose of this study was to clarify the importance of the OATP1B1 haplotype, especially the **1b* allele, in the pharmacokinetics of the OATP1B1 substrates pravastatin, valsartan, and temocaprilat, as well as to determine whether the clearances of OATP1B1 substrate drugs in each subject are well correlated with one another in healthy Japanese volunteers.

METHODS

Subjects. Twenty-three healthy male Japanese volunteers participated in this clinical study. They were recruited from a population of 100 male Japanese volunteers whose OATP1B1 haplotype was prescreened after written informed consent was obtained. The genotyping method of OATP1B1 has been described previously.[10] The haplotypes of OATP1B1 in the 23 participants were **1a/*1a* (n = 5), **1a/*15* (n = 6), **1b/*1b* (n = 7), and **1b/*15* (n = 5). The participants were aged between 20 and 35 years. Each participant had a body weight of between 50 and 80 kg and a body mass index of between 17.6 and 26.4 kg/m^2. Within 1 month before this clinical study was started, a medical history was obtained from the participants, who then underwent a physical examination, electrocardiography, routine blood testing, and urinalysis. They were also screened for narcotic drugs and psychotropic substances. This allowed us to confirm that all of the subjects were able to participate in this study.

Study design. This study protocol was approved by the Ethics Review Boards at both the Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, and Kannondai Clinic, Tsukuba, Japan. All participants provided written informed consent. All subjects took part in the 3-period crossover trial and received pravastatin, valsartan, and temocapril in a random sequence. There was a washout period of 1 week between each administration. In each period subjects came to the clinic on the day before drug administration. After an overnight fast, each subject received 10 mg pravastatin sodium (Mevalotin tablet; Sankyo, Tokyo, Japan), 2 mg temocapril hydrochloride (Acecol tablet; Sankyo), or 40 mg valsartan (Diovan tablet; Novartis, Basel, Switzerland). Venous blood samples (7 mL each) were collected in tubes containing heparin before and at 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, and 24 hours after drug administration. Urine samples were collected for 24 hours. Plasma was separated by centrifugation. Plasma and urine samples were stored at −80°C until analysis. Alcohol, grapefruit juice, St John's wort, and other drugs were not permitted from 2 days before admission to the clinic until the end of the study periods, and smoking was prohibited during the study periods. During the study periods, standardized meals were served to all subjects at scheduled times. For the safety of subjects, after the end of each period, all subjects underwent a physical examination and routine blood testing and urinalysis were carried out.

Quantification of concentrations of pravastatin and its metabolite, RMS-416, in plasma and urine. Concentrations of pravastatin and RMS-416 in plasma and urine were measured by liquid chromatography–tandem mass spectrometry as described in an earlier report.[19] One milliliter of plasma was mixed with 100 μL internal standard (R-122798, 800 ng/mL; prepared by Sankyo), 1 mL 10% methanol, and 300 μL 0.5-mol/L phosphate buffer (pH 4.0). In addition, 0.5 mL urine was mixed with 50 μL internal standard (R-122798), 0.5 mL 10% methanol, and 300 μL 0.5-mol/L phosphate buffer (pH 4.0). The mixture was applied to a Bond Elut C8 cartridge (200 mg/3 mL) (Varian, Palo Alto, Calif), washed twice with 3 mL 5% methanol (plasma) or distilled water (urine), and eluted with 2 mL acetonitrile. The eluate was evaporated under nitrogen gas at 40°C, mixed with 120 μL acetonitrile, and ultrasonicated for 3 minutes. Then, 180 μL 10-mmol/L ammonium acetate was added, and aliquots (20 μL for plasma and 10 μL for urine) were injected into the liquid chromatography–tandem mass spectrometry system. Separation by HPLC was conducted with an Agilent 1100 Series system (Agilent Technologies, Palo Alto, Calif) with an Inertsil ODS-3 column (4.6 × 150 mm, 5 μm; GL Sciences, Tokyo, Japan). The composition of the mobile phase was acetonitrile/water/ammonium acetate/formic acid/triethylamine (400:600:0.77:0.2:0.6 [vol/vol/wt/vol/vol]). The flow rate was 1 mL/min. Mass spectra were determined with an API 4000 tandem mass spectrometer (MDS Sciex, Concord, Ontario, Canada) in the negative ion–detecting mode at the atmospheric pressure–chemical ionization interface. The turbo gas temperature was 600°C. The samples were ionized by reacting with solvent-reactant ions produced by the corona discharge (−5.0 μA) in the chemical ionization mode. The precursor ions of pravastatin at mass-to-charge ratio (*m/z*) 423.2, RMS-416 at *m/z* 423.2, and R-122798 at *m/z* 409.2 were admitted to the first quadrupole (Q1). After the collision-induced fragmentation in the second quadrupole (Q2), the product ions of pravastatin at *m/z* 321.1, RMS-416 at *m/z* 321.3, and R-122798 at *m/z* 321.4 were monitored in the third quadrupole (Q3). The peak area ratio of each compound to the corresponding

internal standard was calculated with Analyst Software (version 1.3.1; Applied Biosystems, Foster City, Calif). The calibration curves were linear over the standard concentration range of 0.1 ng/mL to 100 ng/mL for pravastatin and RMS-416 in plasma, 20 ng/mL to 2000 ng/mL for pravastatin in urine, and 5 ng/mL to 500 ng/mL for RMS-416 in urine.

Quantification of valsartan concentration in plasma and urine. One hundred microliters of plasma or urine was mixed with 100 μL internal standard ($[^2H_9]$-valsartan in 50% methanol, 500 ng/mL; prepared by Novartis Pharma, Basel, Switzerland) and 300 μL 2% trifluoroacetic acid (TFA) aqueous solution. The mixture was applied to a 96-well Empore Disk Plate C18 SD (Sumitomo 3M, Tokyo, Japan); washed 3 times with 200 μL 1% TFA aqueous solution, 1% TFA in 5% methanol, and 1% TFA in 20% methanol; and eluted twice with 100 μL methanol. The eluate was evaporated under nitrogen gas at 40°C, mixed with 100 μL (for plasma) or 400 μL (for urine) methanol/acetonitrile/0.1% TFA (35:20:45 [vol/vol/vol]), and ultrasonicated for 3 minutes. Then, 5-μL aliquots were injected into the liquid chromatography–tandem mass spectrometry system. Separation by HPLC was conducted with an Agilent 1100 Series system (Agilent Technologies) with a Symmetry C18 column (2.1 × 30 mm, 3.5 μm; Waters, Milford, Mass). The composition of the mobile phase was methanol/acetonitrile/0.1% TFA (35:20:45 [vol/vol/vol]). The flow rate was 0.2 mL/min. Mass spectra were determined with an API 4000 tandem mass spectrometer (Applied Biosystems) in the positive ion–detecting mode at the electrospray ionization interface. The turbo gas temperature was 500°C, and the spray voltage was 5500 V. The precursor ions of valsartan at *m/z* 436.1 and $[^2H_9]$-valsartan at *m/z* 445.1 were admitted to the first quadrupole (Q1). After the collision-induced fragmentation in the second quadrupole (Q2), the product ions of valsartan at *m/z* 291.1 and $[^2H_9]$-valsartan at *m/z* 300.1 were monitored in the third quadrupole (Q3). The peak area ratio of each compound to the corresponding internal standard was calculated with Analyst Software (version 1.3.1; Applied Biosystems). The calibration curves were linear over the standard concentration range of 2 ng/mL to 5000 ng/mL for plasma and 20 ng/mL to 5000 ng/mL for urine.

Quantification of temocapril and temocaprilat concentrations in plasma and urine. Two hundred microliters of plasma was mixed with 200 μL internal standard ($[^2H_5]$-temocaprilat, 10 ng/mL; prepared by Sankyo), 2 mL 0.1% formic acid, and 200 μL methanol. Then, 500 μL urine was mixed with 200 μL internal standard ($[^2H_5]$-temocaprilat), 500 μL 0.5% formic acid, and 500 μL methanol. The mixture was applied to a Sep-Pak Vac PS-2 cartridge (200 mg/3 mL) (Waters), washed with twice with 3 mL distilled water, and eluted twice with 3 mL methanol. The eluate was evaporated under nitrogen gas at 45°C, mixed with 280 μL methanol, and ultrasonicated for 3 minutes. Then, 120 μL 0.2% acetic acid was added, and 10-μL aliquots were injected into the liquid chromatography–tandem mass spectrometry system. Separation by HPLC was conducted with an Agilent 1100 Series system (Agilent Technologies) with a Symmetry C18 column (2.1 × 150 mm, 5 μm; Waters). The composition of the mobile phase was methanol/water/acetic acid (700:300:2 [vol/vol/vol]). The flow rate was 0.2 mL/min. Mass spectra were determined with an API 4000 tandem mass spectrometer (Applied Biosystems) in the positive ion–detecting mode at the electrospray ionization interface. The turbo gas temperature was 600°C, and the spray voltage was 5500 V. The precursor ions of temocapril at *m/z* 477.0, temocaprilat at *m/z* 448.9, and $[^2H_5]$-temocaprilat at *m/z* 454.0 were admitted to the first quadrupole (Q1). After the collision-induced fragmentation in the second quadrupole (Q2), the product ions of temocapril at *m/z* 270.0, temocaprilat at *m/z* 269.8, and $[^2H_5]$-temocaprilat at *m/z* 269.9 were monitored in the third quadrupole (Q3). The peak area ratio of each compound to the corresponding internal standard was calculated with Analyst Software (version 1.3.1; Applied Biosystems). The calibration curves were linear over the standard concentration range of 0.5 ng/mL to 200 ng/mL for temocapril and temocaprilat in plasma, 1 ng/mL to 80 ng/mL for temocapril in urine, and 5 ng/mL to 400 ng/mL for temocaprilat in urine.

Uptake study by use of OATP1B1 expression system. The OATP1B1-expressing human embryonic kidney (HEK) 293 cells and vector-transfected control cells have been established previously, and the transport study was carried out as described previously.[5] Tritium-labeled valsartan and unlabeled valsartan were kindly donated by Novartis Pharma, and carbon 14–labeled temocaprilat and unlabeled temocaprilat were donated by Sankyo. Uptake was initiated by the addition of Krebs-Henseleit buffer containing radiolabeled and unlabeled substrates after cells had been washed twice and preincubated with Krebs-Henseleit buffer at 37°C for 15 minutes. The Krebs-Henseleit buffer consisted of 118-mmol/L sodium chloride, 23.8-mmol/L sodium bicarbonate, 4.8-mmol/L potassium chloride, 1.0-mmol/L potassium phosphate [monobasic], 1.2-mmol/L magnesium sulfate, 12.5-mmol/L *N*-[2-hydroxyethyl]piperazine-*N*′-

[2-ethanesulfonic acid] (HEPES), 5.0-mmol/L glucose, and 1.5-mmol/L calcium chloride adjusted to pH 7.4. The uptake was terminated at a designated time by the addition of ice-cold Krebs-Henseleit buffer after removal of the incubation buffer. Cells were then washed twice with 1 mL of ice-cold Krebs-Henseleit buffer, solubilized in 500 μL of 0.2N sodium hydroxide, and kept overnight at 4°C. Aliquots (500 μL) were transferred to scintillation vials after the addition of 250 μL of 0.4N hydrochloric acid. The radioactivity associated with the cells and incubation buffer was measured in a liquid scintillation counter (LS6000SE; Beckman Coulter, Fullerton, Calif) after the addition of 2 mL of scintillation fluid (Clear-sol I; Nacalai Tesque, Kyoto, Japan) to the scintillation vials. The remaining 50 μL of cell lysate was used to determine the protein concentration by the method of Lowry et al[18a] with bovine serum albumin as a standard.

Transcellular transport study by use of double-transfected cells. The transcellular transport study was performed as reported previously by Sasaki et al.[18] In brief, Madin-Darby canine kidney II (MDCKII) cells were grown on Transwell membrane inserts (6.5-mm diameter, 0.4-μm pore size; Corning Coster, Bodenheim, Germany) at confluence for 3 days, and the expression level of transporters was induced with 5-mmol/L sodium butyrate for 2 days before the transport study. Cells were first washed with Krebs-Henseleit buffer at 37°C. Subsequently, substrates were added in Krebs-Henseleit buffer either to the apical compartments (250 μL) or to the basolateral compartments (1 mL). After a designated period, the aliquot of the incubation buffer in the opposite compartments (100 μL from apical compartment or 250 μL from basal compartment) was collected. The amount of tritium-labeled estradiol-17β-glucuronide in the samples was determined by a liquid scintillation counter (LS6000SE; Beckman Coulter), and the amount of temocapril and RMS-416 in the samples was determined by liquid chromatography–mass spectrometry as described later.

Quantification of temocapril concentration in Krebs-Henseleit buffer. A 50-μL sample was mixed vigorously with 250 μL of ethyl acetate. Two hundred microliters of supernatant was collected, dried up by a centrifugal concentrator (TOMY, Tokyo, Japan), and dissolved in 40 μL dimethylsulfoxide. Thirty-microliter aliquots were injected into the liquid chromatography–tandem mass spectrometry system. Separation by HPLC was conducted with a Waters Alliance 2695 Separations Module with an L-column octadecylsilane (2.1 × 150 mm, 5 μm; Chemicals Evaluation and Research Institute, Tokyo, Japan). The composition of the mobile phase was acetonitrile/0.05% formic acid (40:60 [vol/vol]). The flow rate was 0.3 mL/min. Mass spectra were determined with a Micromass ZQ2000 mass spectrometer (Waters) in the positive ion–detecting mode at the electrospray ionization interface. The source temperature and desolvation temperature were 100°C and 350°C, respectively. The capillary, cone, and extractor voltages were 3200 V, 30 V, and 5 V, respectively. The cone gas flow and desolvation gas flow were 65 L/h and 375 L/h, respectively. The mass spectrometer was operated in the selected ion monitoring mode by use of a positive ion, *m/z* 477.30 for temocapril. The retention time of temocapril was approximately 3.7 minutes. Standard curves were linear over the range of 3 to 300 nmol/L.

Quantification of RMS-416 concentration in Krebs-Henseleit buffer. A 60-μL sample was mixed vigorously with 60 μL of methanol including internal standard (0.5 μg/mL R-122798; kindly donated by Sankyo) and deproteinized by centrifugation for 10 minutes at 15,000 rpm at 4°C. Then, 50 μL of supernatant was injected into the liquid chromatography–tandem mass spectrometry system. Separation by HPLC was conducted with a Waters Alliance 2695 Separations Module with an Inertsil ODS-3 column (4.6 × 150 mm, 5 μm; GL Sciences). The composition of the mobile phase was acetonitrile/ammonium acetate, 10 mmol/L (pH 4) (40:60 [vol/vol]). The flow rate was 0.3 mL/min. Mass spectra were determined with a Micromass ZQ2000 mass spectrometer (Waters) in the negative ion–detecting mode at the electrospray ionization interface. The source temperature and desolvation temperature were 100°C and 350°C, respectively. The capillary, cone, and extractor voltages were 3200 V, 20 V and 5 V, respectively. The cone gas flow and desolvation gas flow were 65 L/h and 375 L/h, respectively. The mass spectrometer was operated in the selected ion monitoring mode by use of respective positive ions, *m/z* 423.30 for RMS-416 and *m/z* 409.30 for R-122798 (internal standard). The retention time of RMS-416 and R-122798 was approximately 3.6 minutes and 2.6 minutes, respectively. Standard curves were linear over the range of 5 to 1000 nmol/L.

Pharmacokinetic and statistical analyses. The AUC from time 0 to 24 hours ($AUC_{0\text{-}24}$) was calculated by the linear trapezoidal rule. Renal clearance (CL_r) was calculated by division of the cumulative amount of drug in urine collected for 24 hours by $AUC_{0\text{-}24}$. All pharmacokinetic data are given as mean ± SD. Statistical differences between the data for each haplotype group were determined by ANOVA, followed by the Fisher

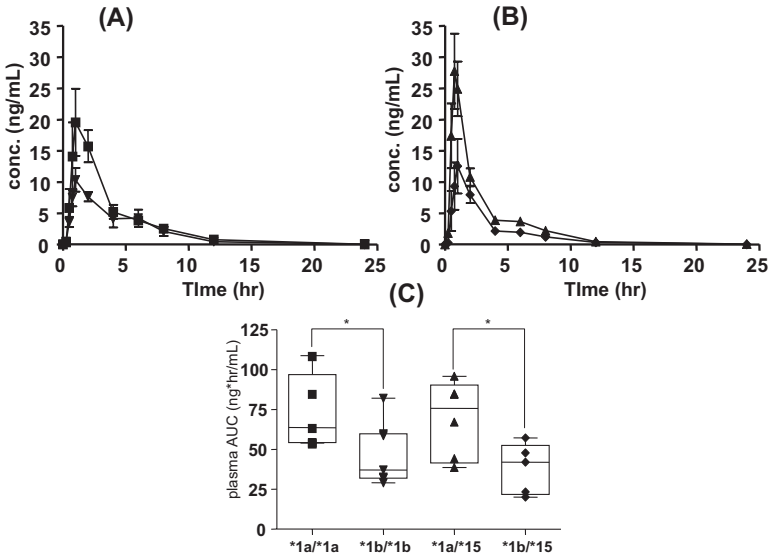

Fig 1. Effect of organic anion transporting polypeptide (OATP) 1B1 haplotype on pharmacokinetics of pravastatin. Plasma concentration (conc)–time profiles of pravastatin after oral administration of 10 mg pravastatin in *OATP1B1∗1a/∗1a* subjects (*squares*, n = 5) and *∗1b/∗1b* subjects (*inverted triangles*, n = 7) (**A**) and in *∗1a/∗15* subjects (*triangles*, n = 6) and *∗1b/∗15* subjects (*diamonds*, n = 5) (**B**). Each *point* represents mean ± SD. **C**, Box-whisker plot of area under plasma concentration–time curve (AUC) of pravastatin in each haplotype group. The *horizontal line* within each *box* represents the median. The *box edges* represent the lower (25th) and upper (75th) quartiles. The *whiskers* extend from the lower and upper quartiles to the furthest data points still within a distance of 1.5 interquartile ranges from the lower and upper quartiles. Individual data points were overlaid on the box-whisker plot. *Asterisk*, Statistically significant difference shown by ANOVA with Fisher least significant difference test ($P < .05$).

least significant difference test. $P < .05$ was considered to be statistically significant.

RESULTS

Effect of OATP1B1 haplotype on pharmacokinetics of pravastatin and its metabolite, RMS-416. After oral administration of pravastatin, the plasma concentration of pravastatin in *OATP1B1∗1b/∗1b* subjects was lower than that in *∗1a/∗1a* subjects (Fig 1, *A*). Similarly, the plasma concentration in *∗1b/∗15* subjects was lower than that in *∗1a/∗15* subjects (Fig 1, *B*). The mean $AUC_{0\text{-}24}$ of pravastatin in *∗1b/∗1b* subjects was significantly lower than that in *∗1a/∗1a* subjects (65% of *∗1a/∗1a*), and the $AUC_{0\text{-}24}$ in *∗1b/∗15* subjects was significantly lower than that in *∗1a/∗15* subjects (55% of *∗1a/∗15*) (Fig 1, *C*, and Table I). In addition, CL_r was not significantly different among the haplotype groups (Table I). Pravastatin was converted to RMS-416 by chemical epimerization. We also calculated the concentration of the sum of pravastatin and RMS-416 in plasma and urine. The $AUC_{0\text{-}24}$ value of the sum of pravastatin and RMS-416 in *∗1b* carriers tended to be lower than that in *∗1a* carriers, whereas this value in *∗15* carriers tended to be higher than that in non-*∗15* carriers (Table I). The CL_r calculated from the sum of pravastatin and RMS-416 was not markedly different between each haplotype group.

Effect of OATP1B1 haplotype on pharmacokinetics of valsartan. After oral administration of valsartan, the plasma concentration of valsartan in *OATP1B1∗1b/∗1b* subjects was lower than that in *∗1a/∗1a* subjects (Fig 2, *A*) and the plasma concentration in *∗1b/∗15* subjects was lower than that in *∗1a/∗15* subjects (Fig 2, *B*). Although the difference did not reach statistical significance, the mean $AUC_{0\text{-}24}$ of valsartan in *∗1b/∗1b* subjects tended to be lower than that in *∗1a/∗1a* subjects (73% of *∗1a/∗1a*), and the $AUC_{0\text{-}24}$ in *∗1b/∗15* subjects was significantly lower than that in *∗1a/∗15* subjects (67% of *∗1a/∗15*) (Fig 2, *C*, and Table I), exhibiting a trend similar to pravastatin. The CL_r was almost the same in each haplotype group (Table I).

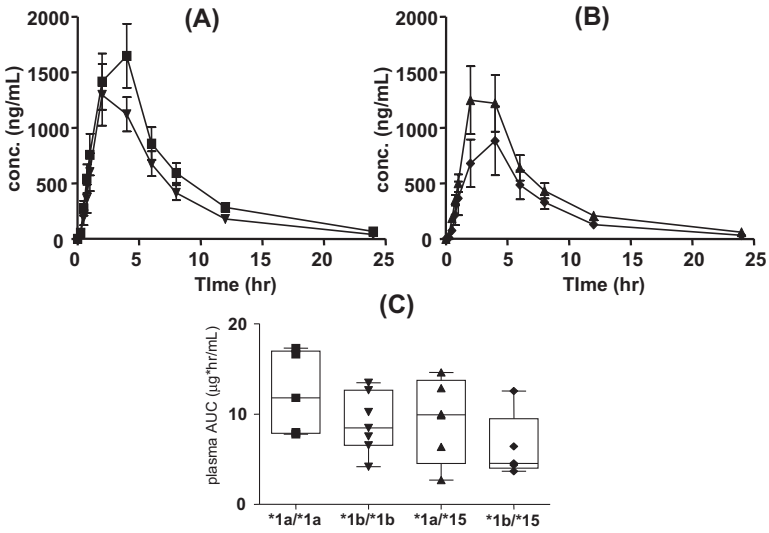

Fig 2. Effect of OATP1B1 haplotype on pharmacokinetics of valsartan. Plasma concentration–time profiles of valsartan after oral administration of 40 mg valsartan in *OATP1B1∗1a/∗1a* subjects (*squares*, n = 5) and *∗1b/∗1b* subjects (*inverted triangles*, n = 7) (**A**) and in *∗1a/∗15* subjects (*triangles*, n = 6) and *∗1b/∗15* subjects (*diamonds*, n = 5) (**B**). **C**, Box-whisker plot of AUC of valsartan in each haplotype group.

Table I. $AUC_{0\text{-}24}$ and CL_r of pravastatin and its metabolite (RMS-416), valsartan, and temocapril and its active metabolite (temocaprilat) in each haplotype group

	∗1a/∗1a (n = 5)	*∗1b/∗1b (n = 7)*	*∗1a/∗15 (n = 6)*	*∗1b/∗15 (n = 5)*
Pravastatin				
$AUC_{0\text{-}24}$ (ng · h/mL)	73.2 ± 23.5	47.4 ± 19.9†	69.2 ± 23.4	38.2 ± 15.9‡
CL_r (L/h)	15.1 ± 2.7	18.6 ± 7.6	12.6 ± 2.5	17.0 ± 3.5
Pravastatin plus RMS-416				
$AUC_{0\text{-}24}$ (ng · h/mL)	112 ± 25	76.1 ± 20.4	143 ± 40	95.1 ± 33.6‡
CL_r (L/h)	12.5 ± 2.0	16.0 ± 8.5	11.0 ± 1.5	13.5 ± 2.4
Valsartan				
$AUC_{0\text{-}24}$ (μg · h/mL)	12.3 ± 4.6	9.01 ± 3.33	9.40 ± 4.34	6.31 ± 3.64
CL_r (L/h)	0.450 ± 0.063	0.482 ± 0.049	0.489 ± 0.109	0.477 ± 0.096
Temocaprilat				
$AUC_{0\text{-}24}$ (ng · h/mL)	426 ± 91	371 ± 100	429 ± 41	389 ± 77
CL_r (L/h)	1.41 ± 0.06	1.29 ± 0.26	1.31 ± 0.12	1.32 ± 0.15
Temocapril				
$AUC_{0\text{-}24}$ (ng · h/mL)	18.5 ± 7.7	12.4 ± 4.1	19.0 ± 4.1	16.4 ± 5.0
CL_r (L/h)	0.818 ± 0.476	1.82 ± 1.16	1.21 ± 0.52	2.14 ± 1.77

Data are presented as mean ± SD.
$AUC_{0\text{-}24}$, Area under plasma concentration–time curve from 0 to 24 hours; CL_r, renal clearance.
†Significantly different from values in ∗1a/∗1a subjects as determined by ANOVA with Fisher least significant difference test ($P < .05$).
‡Significantly different from values in ∗1a/∗15 subjects as determined by ANOVA with Fisher least significant difference test ($P < .05$).

Effect of OATP1B1 haplotype on pharmacokinetics of temocapril and temocaprilat. After oral administration of temocapril, temocapril was rapidly eliminated from the blood, and the concentration of temocapril was undetectable at 1 to 2 hours after administration as a result of the rapid conversion of temocapril to the active metabolite temocaprilat by carboxylesterase. Temocaprilat was then detected at 0.25 hour, and its plasma concentration reached a maximum at 0.75 to 2 hours after intake of temocapril (Fig 3, *A* and *B*). The plasma concentration of

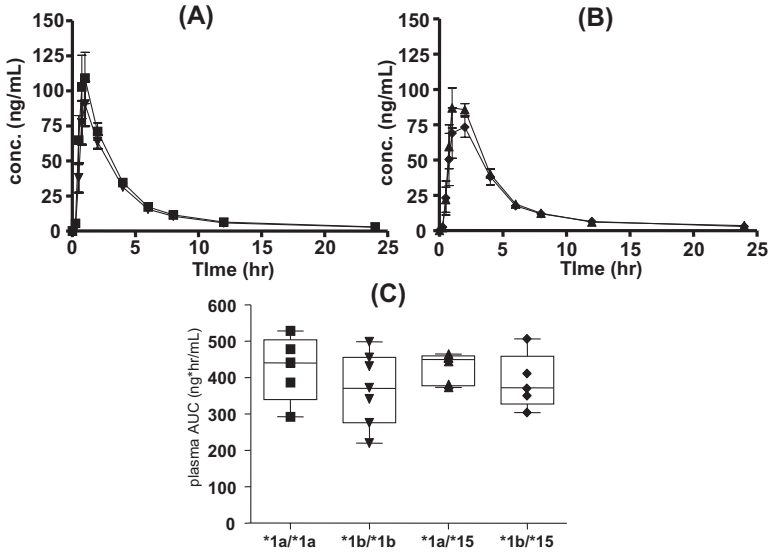

Fig 3. Effect of OATP1B1 haplotype on pharmacokinetics of temocaprilat. Plasma concentration–time profiles of temocaprilat after oral administration of 2 mg temocapril in *OATP1B1∗1a/∗1a* subjects (*squares*, n = 5) and *∗1b/∗1b* subjects (*inverted triangles*, n = 7) (**A**) and in *∗1a/∗15* subjects (*triangles*, n = 6) and *∗1b/∗15* subjects (*diamonds*, n = 5) (**B**). **C**, Box-whisker plot of AUC of temocaprilat in each haplotype group.

temocaprilat showed a similar pattern in *OATP1B1∗1b/∗1b* and *∗1a/∗1a* subjects (Fig 3, *A*) and *∗1b/∗15* and *∗1a/∗15* subjects (Fig 3, *B*). The mean $AUC_{0\text{-}24}$ of temocaprilat in *∗1b/∗1b* subjects was not significantly very different from that in *∗1a/∗1a* subjects (87% of *∗1a/∗1a*), and the $AUC_{0\text{-}24}$ in *∗1b/∗15* subjects was not different from that in *∗1a/∗15* subjects (91% of *∗1a/∗15*) (Fig 3, *C* and Table I). The *OATP1B1∗15* allele did not affect the $AUC_{0\text{-}24}$ of temocaprilat. The CL_r of temocaprilat was almost the same in each haplotype group (Table I). On the other hand, the plasma concentration of temocapril in *OATP1B1∗1b/∗1b* subjects tended to be lower than that in *∗1a/∗1a* subjects (Fig 4, *A*), and the plasma concentration in *∗1b/∗15* subjects tended to be lower than that in *∗1a/∗15* subjects (Fig 4, *B*). Although not statistically significant, the $AUC_{0\text{-}24}$ of temocapril in *∗1b/∗1b* carriers was lower than that in *∗1a/∗1a* carriers (67% of *∗1a/∗1a*) and the $AUC_{0\text{-}24}$ in *∗1b/∗15* carriers was lower than that in *∗1a/∗15* carriers (86% of *∗1a/∗15*) (Fig 4, *C*, and Table I), and the CL_r of temocapril in each haplotype group was not significantly different (Table I).

Correlation between AUC of pravastatin, valsartan, temocaprilat, and temocapril in each subject. The AUC values of valsartan in each subject were significantly correlated with those of pravastatin ($R = 0.630$, $P < .01$) (Fig 5, *A*). The AUC values of temocapril in each subject were also significantly correlated with those of pravastatin ($R = 0.602$, $P < .01$) (Fig 5, *C*). However, the AUC values of temocaprilat were not significantly correlated with those of pravastatin ($R = 0.229$) (Fig 5, *B*).

OATP1B1-mediated uptake of valsartan and temocaprilat in expression system. The time-dependent uptake of valsartan and temocaprilat in OATP1B1-expressing HEK293 cells was significantly higher than that in vector-transfected control cells (Fig 6).

OATP1B1-/MRP2-mediated transcellular of temocapril and RMS-416 in OATP1B1/MRP2 double-transfected MDCKII cells. As a positive control, we ascertained that the vectorial basal-to-apical transcellular transport of estradiol-17β-glucuronide (OATP1B1/MRP2 bisubstrate) was clearly observed in OATP1B1/MRP2 double-transfected cells (Fig 7, *C*), whereas symmetric transport was observed in OATP1B1 single-transfected cells and vector-transfected control cells (Fig 7, *A* and *B*) as shown in the previous report.[18] Under the same condition, basal-to-apical transport of temocapril and RMS-416 was significantly larger than that in the opposite direction in OATP1B1/MRP2 double-transfected cells (Fig 7, *F* and *I*), whereas their vectorial transport was not observed in OATP1B1 single-transfected cells (Fig 7, *E* and *H*) and vector-transfected control cells (Fig 7, *D* and *G*).

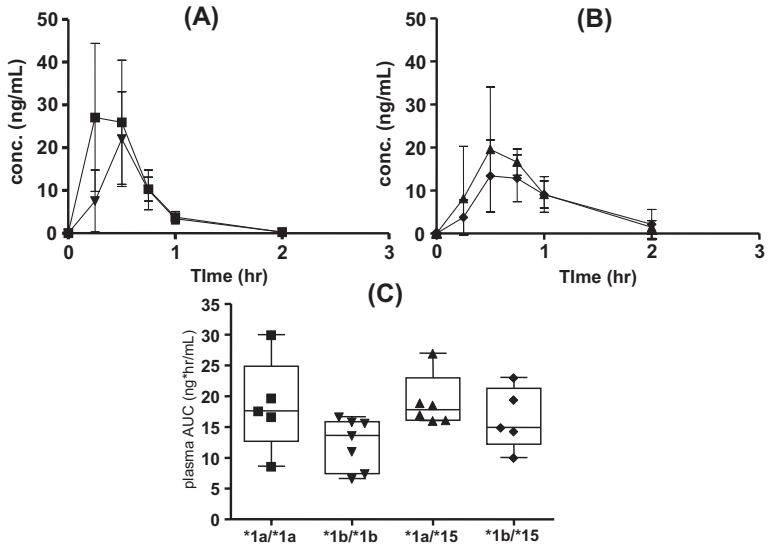

Fig 4. Effect of OATP1B1 haplotype on pharmacokinetics of temocapril. Plasma concentration–time profiles of temocapril after oral administration of 2 mg temocapril in *OATP1B1∗1a/∗1a* subjects (*squares*, n = 5) and *∗1b/∗1b* subjects (*inverted triangles*, n = 7) (**A**) and in *∗1a/∗15* subjects (*triangles*, n = 6) and *∗1b/∗15* subjects (*diamonds*, n = 5) (**B**). **C**, Box-whisker plot of AUC of temocapril in each haplotype group.

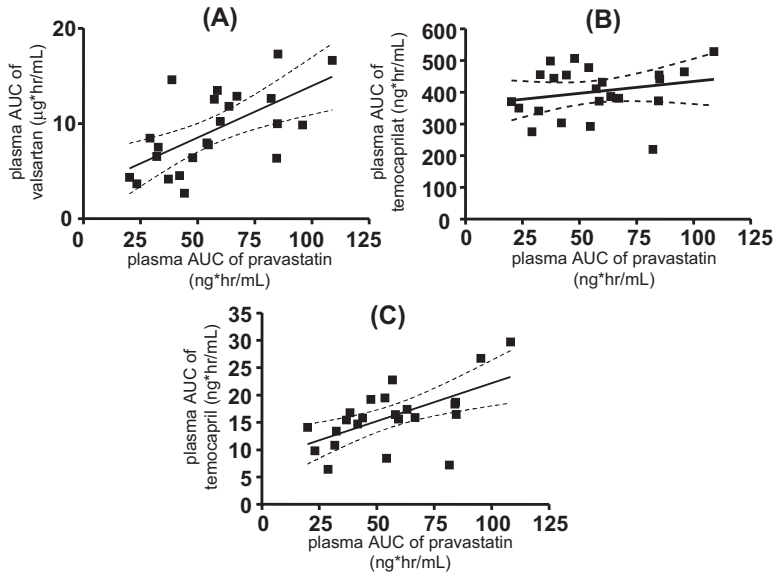

Fig 5. Correlation between AUC of pravastatin and valsartan (**A**), AUC of pravastatin and temocaprilat (**B**), and AUC of pravastatin and temocapril (**C**). Each *point* represents data for each subject. *Solid lines* represent fitted lines calculated by linear regression analysis, and *dotted lines* represent 95% confidence intervals of correlations.

DISCUSSION

We investigated the impact of the OATP1B1 haplotype, especially *∗1b*, on the pharmacokinetics of 3 anionic drugs, pravastatin, valsartan, and temocapril, in healthy volunteers. We also investigated whether the pharmacokinetics of each drug was correlated with that of other drugs in each subject.

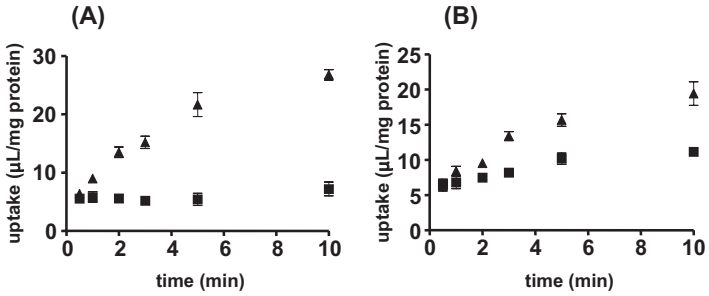

Fig 6. Time-dependent uptake of valsartan (**A**) and temocaprilat (**B**) in OATP1B1-expressing cells and control cells. The uptake of valsartan and temocaprilat by OATP1B1-transfected human embryonic kidney (HEK) 293 cells was examined at 37°C. *Triangles* and *squares* represent uptake by OATP1B1- and vector-transfected cells, respectively. Each *point* represents mean ± SE (n = 3).

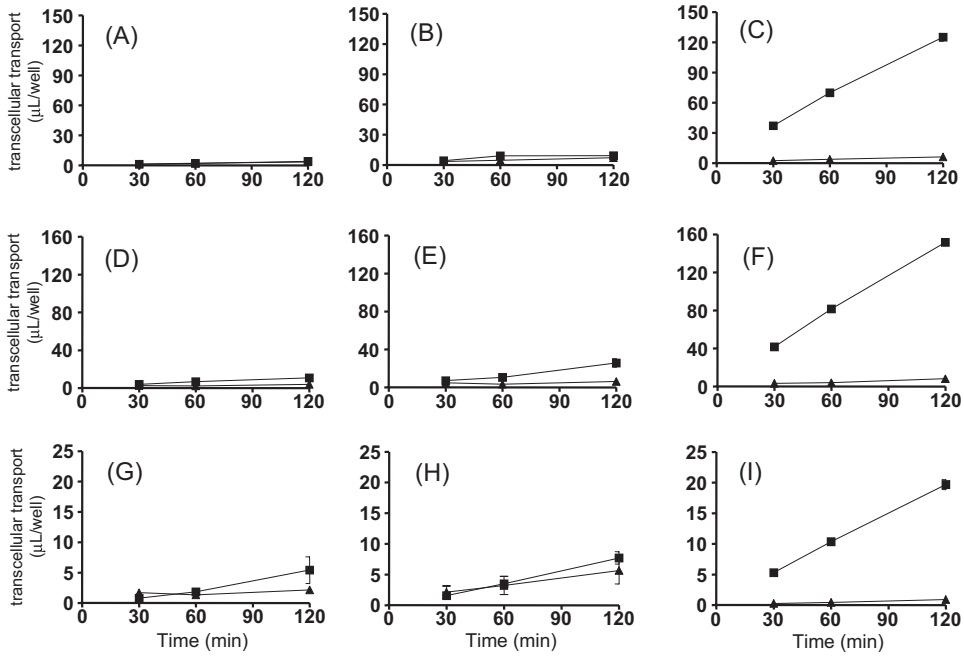

Fig 7. Time profiles for transcellular transport of estradiol-17β-glucuronide (**A**, **B**, and **C**), temocapril (**D**, **E**, and **F**), and RMS-416 (**G**, **H**, and **I**) across Madin-Darby canine kidney II (MDCKII) monolayers. Transcellular transport of estradiol-17β-glucuronide (1 μmol/L), temocapril (1 μmol/L), and RMS-416 (10 μmol/L) across MDCKII monolayers expressing OATP1B1 (**B**, **E**, and **H**) and both OATP1B1 and multidrug resistance associated protein 2 (MRP2) (**C**, **F**, and **I**) was compared with that across the control MDCKII monolayer (**A**, **D**, and **G**). *Triangles* and *squares* represent transcellular transport in apical-to-basal direction and basal-to-apical direction, respectively. Each *point* and *vertical bars* represent mean ± SE of 3 determinations. Where vertical bars are not shown, the SE was contained within the limits of the symbol.

Pravastatin, valsartan, and temocaprilat are mainly excreted into the bile without extensive metabolism, and the involvement of transporters is needed to explain their efficient accumulation in the liver. When the pharmacokinetic theory is considered, given the assumption that total clearance is accounted for by hepatic clearance, the AUC after oral administration of drugs (AUC_{oral}) can be expressed by equation 1:

$$AUC_{oral} = \frac{F \cdot Dose}{f_B \cdot CL_{int}} \quad (1)$$

in which F is the fraction of the dose absorbed into and through the gastrointestinal membranes, f_B is the blood unbound fraction, and CL_{int} is the hepatic intrinsic clearance. In addition, CL_{int} can be expressed by equation 2:

$$CL_{int} = CL_{uptake} \cdot \frac{CL_{ex} + CL_{metab}}{CL_{ex} + CL_{metab} + CL_{eff}} \quad (2)$$

where CL_{uptake}, CL_{ex}, CL_{metab}, and CL_{eff} represent the intrinsic clearances for hepatic uptake, biliary excretion, metabolism, and sinusoidal efflux, respectively.[6] When equations 1 and 2 are considered, CL_{uptake} directly affects the AUC after oral administration in any situation.

We especially focused on the impact of the *OATP1B1∗1b* allele on the pharmacokinetics of the 3 drugs. The allele frequency of *OATP1B1∗1b* is relatively high in some ethnic groups: 0.30 in white Americans, 0.74 in black Americans, and 0.63 in Japanese subjects.[9,10]

Pravastatin has been reported to be a substrate of OATP1B1.[1,8,18,20] We demonstrated that valsartan and temocaprilat could also be recognized by OATP1B1 as substrates (Fig 6).

The *OATP1B1∗1b* allele significantly reduced the AUC of pravastatin compared with *∗1a*, which is consistent with a previous report.[12] The CL_r of pravastatin was not affected by *∗1b*, suggesting that the *OATP1B1∗1b* variant apparently enhances the hepatic uptake activity of pravastatin. On the other hand, the *OATP1B1∗15* allele did not remarkably affect the AUC of pravastatin in our study, which apparently differs from findings in earlier reports showing that the *∗15* allele results in a significant increase in the AUC of pravastatin.[10,11] After oral administration, a significant amount of pravastatin was converted to RMS-416 (3′α-isopravastatin), mainly in the stomach, by a chemical reaction rather than by enzymatic metabolism, because pravastatin is unstable in acidic solution.[21] In our study the interindividual difference in the AUC of RMS-416 was about 50-fold (2.37-120 ng · h/mL; mean, 48.8 ng · h/mL). It is generally accepted that the pH values in the stomach exhibit large interindividual differences. We hypothesized that these differences might affect the conversion rate to RMS-416, which could mask the effect of *OATP1B1∗15* on the pharmacokinetics of pravastatin in our cases. RMS-416 is an epimer of pravastatin, and only the position of 1 hydroxyl group was different. We confirmed that RMS-416 is also a substrate of OATP1B1 (Fig 7). If it is assumed that the pharmacokinetic profile of pravastatin was not so different from that of RMS-416 because of their similar chemical structures, a genetic polymorphism of OATP1B1 would affect the pharmacokinetics of the sum of pravastatin and RMS-416 more markedly than that of pravastatin itself. In our study we could see the expected tendency showing that the *∗1b* allele reduced the AUC of the sum of pravastatin and RMS-416 compared with *∗1a*, whereas the *∗15* allele increased the AUC (Table I). Recent studies have demonstrated that the Val174Ala mutation in OATP1B1 could alter the cholesterol-lowering effect of pravastatin.[22,23] Because RMS-416 is not pharmacologically active, our study suggests that the conversion rate to RMS-416, as well as the genetic polymorphism in OATP1B1, may have an effect on the pharmacokinetics and pharmacologic action of pravastatin.

In the case of valsartan the *∗1b* allele showed a reduction in the AUC of valsartan, but the *∗15* allele did not show any increase in the AUC, which is almost the same as pravastatin. Unfortunately, the difference in its AUC between *∗1a* and *∗1b* did not show statistical significance, probably because of the lack of statistical power, and we believe that a greater number of subjects will be needed to show the significant difference. Valsartan was partly metabolized to the 4-hydroxylated form (M-2) by CYP2C9,[24] but a previous report indicated that, after oral administration of [^{14}C]-valsartan, this metabolite accounted for only 10% of the total radioactivity in the feces and urine.[24] Therefore the interindividual difference in CYP2C9 activity plays only a minor role in the pharmacokinetics of valsartan. The solubility of valsartan is drastically affected by the pH, and it is possible that an interindividual difference in the pH value in the gastrointestinal tract may affect its solubility and the subsequent amount of valsartan absorbed.[25] However, 1 report demonstrated that coadministration of cimetidine increased the AUC only by 7%.[26] Thus the exact reason why the *∗15* allele did not affect the pharmacokinetics of valsartan remains to be clarified.

The AUC of temocapril and temocaprilat was not changed significantly in each haplotype group. However, there was a trend suggesting that the *∗1b* allele reduced the AUC of temocapril and temocaprilat and that the *∗15* allele showed a slight increase in the AUC. We determined that temocapril is also a substrate of OATP1B1 (Fig 7). The difference in the AUC of temocaprilat in each haplotype group was relatively small compared with that of the other drugs.

We also compared the pharmacokinetics of the 3 drugs in each subject, and the AUC of pravastatin was

significantly correlated with that of valsartan and temocapril, but not temocaprilat (Fig 5). This result suggested that the clearance mechanism of pravastatin may be shared with that of valsartan and temocapril and that the relative contribution of OATP1B1 to the hepatic uptake of pravastatin may be similar to that of valsartan and temocapril but larger than that of temocaprilat.

Some in vitro studies have indicated that the transport activity of several compounds including pravastatin in the *OATP1B1∗1b* variant was almost comparable to that of *OATP1B1∗1a*.[7–9,27] However, this study demonstrated that *OATP1B1∗1b* subjects showed an increase in hepatic clearance compared with that of *OATP1B1∗1a* subjects. We hypothesized that this apparent discrepancy may be explained by the higher expression level of *OATP1B1∗1b* in the liver compared with that of *OATP1B1∗1a*. This can be proven by investigating the relative expression level of *OATP1B1∗1a* and *∗1b* in several batches of human hepatocytes that are genotyped in advance. Moreover, we must pay attention to several pharmacokinetic issues such as the different proportion of hepatic clearance to total clearance, the different contribution of OATP1B1 to overall hepatic uptake, and the substrate specificity of the effect of genetic polymorphisms in OATP1B1. The percentage of hepatic clearance with regard to total clearance has been estimated to be about 53% for pravastatin, 71% for valsartan, and 62% for temocaprilat in humans[24,28] (drug information for temocaprilat provided by Sankyo). Previous reports suggest that the de-esterification of temocapril mainly occurs in the liver (drug information published by Sankyo). The urinary excretion of temocapril as a percentage of the administered dose is about 1.1% in this study. We believe that it is possible that temocapril is efficiently taken up into hepatocytes, followed by conversion to temocaprilat, and its hepatic clearance is much higher than its CL_r. That may be the reason why the AUC of temocapril showed a better correlation with that of pravastatin than with that of temocaprilat. Regarding the contribution of individual transporters, our preliminary study suggested that all 3 compounds are substrates of OATP1B3, as well as OATP1B1 (Hirano M, Maeda K, Sugiyama Y, unpublished data, Aug 25, 2004). The previous studies suggested that pravastatin is thought to be mainly taken up via OATP1B1.[20] From the result of the present study, we speculated that the relative importance of OATP1B1 with regard to hepatic clearance is in the following order: pravastatin greater than valsartan and temocapril greater than temocaprilat. We have established the method for estimating the contribution of OATP1B1 and OATP1B3 to overall hepatic uptake by using expression systems and human hepatocytes.[5] With this information being taken into consideration, the prediction of the effect of genetic polymorphisms in OATP1B1 on the pharmacokinetics of drugs from in vitro data will be the subject of further investigation.

In conclusion, our study indicated that the *OATP1B1∗1b* allele increases the hepatic clearance of pravastatin compared with that of the *∗1a* allele, and valsartan and temocapril showed a similar tendency. In addition, the AUC of pravastatin correlates well with that of valsartan and temocapril, suggesting that pravastatin, valsartan, and temocapril may share the same elimination pathway.

We thank Sankyo for providing Mevalotin tablets, Acecol tablets, [^{14}C]-labeled and unlabeled temocaprilat, temocapril, RMS-416, and R-122798 and Novartis Pharma for providing Diovan tablets and tritium-labeled and unlabeled valsartan. We are grateful to Dr Kiyoshi Kawabata (Sankyo) for teaching us the method for the quantification of pravastatin, RMS-416, temocapril, and temocaprilat. We are also grateful to Drs Ryosei Kawai and Yuko Tsukamoto, Novartis Pharma, and Drs Toshihiko Ikeda, Yasushi Orihashi, and Hideo Naganuma, Sankyo, for supporting our study and giving us helpful suggestions.

The authors have no conflict of interest.

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