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Impact of CYP3A5 genotype on tolvaptan pharmacokinetics and their relationships with endogenous markers of CYP3A activity and serum sodium level in heart failure patients

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Abstract

Tolvaptan efficacy for heart failure has a large interindividual variation. This study aimed to evaluate the influence of CYP3A5 and ABCB1 genotypes on tolvaptan pharmacokinetics and their relationships with plasma markers of CYP3A activity and laboratory test values in heart failure patients. Fifty-eight heart failure patients receiving oral tolvaptan for volume overload were enrolled. Blood samples for determination of predose plasma concentrations of tolvaptan and its metabolites were collected. CYP3A5 and ABCB1 genotypes, plasma 4β-hydroxycholesterol/total cholesterol ratio (4β-OHC/TC) and 25-hydroxyvitamin D (25-OHD), and serum laboratory test values were evaluated. The *CYP3A5*3/*3* genotype was associated with a higher plasma concentration of tolvaptan but not with its metabolic ratios. The *ABCB1 3435C>T*, *2677G>T/A*, and *1236C>T* polymorphisms affected neither tolvaptan pharmacokinetics nor its metabolism. Plasma 4β-OHC/TC and 25-OHD concentration were not correlated with plasma tolvaptan concentration. In a stratified analysis based on CYP3A5 genotype, plasma 4β-OHC/TC had a negative correlation with plasma tolvaptan concentration in the patients with the *CYP3A5*1* allele, while the plasma concentration of 25-OHD did not. The *CYP3A5*3/*3* genotype was associated with a higher serum sodium level in the patients with volume overload. The plasma concentration of 25-OHD had a positive correlation with the serum total bilirubin level. In conclusion, *CYP3A5*3* but not ABCB1 genotypes elevated tolvaptan plasma exposure in heart failure patients. CYP3A5-deficient patients treated with tolvaptan had a higher serum sodium level. The CYP3A5 genotype altered the relationship

between plasma tolvaptan and 4β-OHC.

Introduction and background

Tolvaptan, an orally effective vasopressin V_2 receptor antagonist, raises excretion of electrolyte-free water by competitively binding to the receptor on the renal collecting ducts [1]. Oral tolvaptan has a large interindividual variation in intestinal absorption [2]. In heart failure patients, tolvaptan plasma exposure and its diuretic effect also showed large differences [3]. Adverse effects such as severe hypernatremia and potentially fatal liver injury were reported in clinical settings [4,5]. However, tolvaptan plasma exposure itself has not fully explained the efficacy and safety [6].

Tolvaptan is mainly converted to three monohydroxylates (DM-4110, DM-4111, and DM-4119) and a ring-opened carboxylate (DM-4103) in the human liver (Figure S1) [7]. Each monohydroxylate possesses 2- to 4-fold less activity to inhibit V_2 receptors than tolvaptan [8]. DM-4103 inhibits hepatic bile acid transport proteins such as organic anion transporting polypeptide (OATP) 1B1 and bile salt export pump, and is likely involved in the induction of liver injury [9]. Tolvaptan and its metabolites are predominantly eliminated by hepatic CYP3A4/5 in humans [7]. In addition, tolvaptan is a substrate of and a weak inhibitor of P-glycoprotein, ABCB1 transporter, which is involved in the intestinal secretion and multidrug resistance [10].

CYP3A activity varies among human individuals. CYP3A5 frequently possesses a single nucleotide polymorphism (SNP) affecting its activity, which is known as *CYP3A5*3* allele [11]. The *CYP3A5*3/*3* genotype, which is characterized as the almost complete absence

of CYP3A5 protein, decreases drug clearances of CYP3A5 substrates such as tacrolimus and fentanyl in patients [12,13]. P-glycoprotein also has several SNPs affecting its transport activity [14]. The *ABCB1 3435TT* genotype elevates the plasma exposures of tacrolimus and digoxin through increased intestinal absorption in humans [15,16]. The contributions of the CYP3A5 and ABCB1 genotypes to tolvaptan pharmacokinetics remain to be clarified in humans.

Plasma 4β-hydroxycholesterol (4β-OHC), a cholesterol CYP3A-mediated metabolite, has recently emerged as a promising endogenous marker of CYP3A activity in humans. Plasma 4β-OHC as a non-P-glycoprotein activity marker has been utilized in a number of biomarker studies for predicting the pharmacokinetics of CYP3A probes and influences of CYP3A inducers [17,18]. Plasma 4β-OHC is also useful for the discrimination of patients with *CYP3A5*3/*3* [13]. Plasma 25-hydroxyvitamin D (25-OHD) is another promising biomarker of CYP3A activity. Vitamin D eventually contributes to the increase in mRNA expression and activity level of CYP3A, leading to high metabolism of CYP3A substrates [19]. However, the usefulness of these two endogenous biomarkers for the prediction of plasma tolvaptan and its metabolites has not been evaluated in humans.

No clinical studies evaluating the genetic impact on tolvaptan pharmacokinetics using CYP3A activity markers have been reported. This study aimed to evaluate the influence of CYP3A5 and ABCB1 genotypes on plasma tolvaptan and its metabolites and their relationships with plasma markers of CYP3A activity, serum levels of electrolytes, and liver function test results in heart failure patients.

Materials and Methods

Ethics

This study was conducted in accordance with the ethical standards of the Ethics Committee of Hamamatsu University School of Medicine (17-012) and with the Declaration of Helsinki and its later amendments. The present study was registered as an observation study at a single site (Hamamatsu University Hospital, Hamamatsu, Japan) in the University Hospital Medical Information Network (UMIN000033065). The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies [20]. The patients received information about the scientific aim of the study and each patient provided written informed consent.

Patients and blood sampling

A total of 78 Japanese inpatients treated with a fixed-dose of tolvaptan tablet (Samsca, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) once daily after breakfast during at least six consecutive days for heart failure with volume overload were recruited and 58 were enrolled in this study. Exclusion criteria were as follows: patients who (1) were receiving > 15 mg of tolvaptan per day; (2) were suspected of poor drug adherence based on pharmacist interviews and medical records; (3) were being co-treated with potent CYP3A or ABCB1 inducers and inhibitors including carbamazepine, rifampicin, triazole antifungal agents, macrolide

antibiotics, cyclosporine, tacrolimus, quinidine, digoxin, or verapamil [21]; (4) had an active infectious disease; (5) had liver dysfunction (serum total bilirubin > 2.0 mg/dL) before the initiation of tolvaptan treatment; and (6) were using vitamin D supplementation or subcutaneous denosumab. The blood samples were collected just before dosing on the 7th day after starting tolvaptan treatment or later.

Determination of plasma tolvaptan and its metabolites

Plasma concentrations of tolvaptan and its metabolites including DM-4110, DM-4111, DM-4119, and DM-4103 (Otsuka Pharmaceutical Co., Ltd.) were simultaneously quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) [22]. The linearities (r > 0.999) of tolvaptan, DM-4110, DM-4111, DM-4119, and DM-4103 were observed at plasma concentration ranges of 3.125–1000, 0.3125–100, 1.25–400, 0.625–200, and 31.25–10,000 ng/mL, respectively. The intra- and inter-day accuracies of all the analytes had ranges of 91.6–106.5% and 94.1–105.1%, while their intra- and inter-day imprecisions were < 2.5% and 10.9%, respectively. The lower limits of quantification (LLOQs) for tolvaptan, DM-4110, DM-4111, DM-4119, and DM-4103 were 3.125, 0.3125, 1.25, 0.625, and 31.25 ng/mL, respectively.

Plasma exposure parameters of tolvaptan

Variation in plasma exposure of tolvaptan and its metabolites was assessed as dose and body weight-adjusted plasma concentrations unless otherwise stated. Absolute plasma concentrations

of tolvaptan and its metabolites were used for analysis of their relationship with laboratory test values. Tolvaptan metabolism was evaluated as the metabolic ratio of tolvaptan: the plasma concentration ratio of a metabolite to tolvaptan.

CYP3A5 and ABCB1 genotypings

Genomic DNA was extracted from the whole blood sample of each patient using a DNA Extractor WB Kit (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) and then stored at -20°C until genotyping. The SNPs rs776746 (*CYP3A5*3*), rs1045642 (*ABCB1 3435C>T*), rs2032582 (*ABCB1 2677G>T/A*), and rs1128503 (*ABCB1 1236C>T*) were genotyped by a TaqMan real-time polymerase chain reaction method (Thermo Fisher Scientific, Waltham, MA, USA). The patients with *CYP3A5*1/*1* or **1/*3* were integrated into a group of patients with *CYP3A5*1* allele in the analysis unless otherwise stated. The patients were classified into three genotype groups of *CC*, *CT*, and *TT* for *ABCB1 3435C>T* and *1236C>T*. As for *ABCB 2677G>T/A*, the patients were classified into three genotype groups of *2677GG*; *GT* or *GA*; *TT*, *TA*, or *AA*. The homozygous variants of the three ABCB1 genotypes (*3435TT-2677TT-1236TT*) were defined as ABCB1 triple *TT* diplotype. The effect of the CYP3A5 genotype and the ABCB1 triple *TT* diplotype on plasma tolvaptan pharmacokinetics were evaluated between genders.

Determination of plasma markers of CYP3A activity

Saponified 4β-OHC in plasma was quantified by an LC-MS/MS method [23]. The linearity of 4β-OHC was observed at a plasma concentration range of 5–200 ng/mL ($r > 0.999$). The intra- and inter-day accuracies of 4β-OHC were 104.2–114.5% and 94.5–106.8%, while its intra- and inter-day imprecisions were < 9.3% and 13.6%, respectively. The LLOQ for plasma 4β-OHC was 5 ng/mL. Plasma total cholesterol was measured by an enzymatic method using a Cholesterol E-Test Wako kit (FUJIFILM Wako Pure Chemical Corporation). The LLOQ for plasma total cholesterol was 2.2 mg/dL. Plasma 25-OHD was determined by enzyme-linked immunosorbent assay using a 25(OH)-Vitamin D direct day kit (Immundiagnostik AG, Bensheim, Germany). The LLOQ for plasma 25-OHD was 6.1 ng/mL.

Relationships with laboratory test values

The common laboratory test values of each patient at the point of blood sampling for determination of plasma concentrations of tolvaptan and its metabolites were obtained from their medical records. The absolute plasma concentrations of tolvaptan and its metabolites, the CYP3A5 and ABCB1 genotypes, and the plasma markers of CYP3A activity were assessed with respect to their relationship with serum levels of electrolytes: sodium, potassium, and chloride, serum liver function test results: aspartate aminotransferase, alanine aminotransferase, and total bilirubin, and serum albumin.

Multivariate analyses for tolvaptan pharmacokinetics

Multivariate regression models for the dose and body weight-adjusted plasma concentrations of tolvaptan and its metabolites were built using the stepwise approach in the selection of explanatory variables: gender (male versus female as the reference), age, CYP3A5 genotype (**1* allele carriers versus **3/*3* as the reference), ABCB1 genotype (triple *TT* diplotype versus the others as the reference), plasma 4β-OHC/TC, plasma 25-OHD, serum albumin, serum total bilirubin, and estimated glomerular filtration rate. The estimated glomerular filtration rate was calculated using the Modification of Diet in Renal Disease study equation [24] and the Du Bois formula [25]. The partial regression coefficient was represented as *b*.

Statistical analysis

All statistics were analysed using Stata version 13.0 (StataCorp LLC, College station, TX, USA). Fisher's exact probability test was used for categorical variables. The Kruskal-Wallis test and the *post hoc* Mann-Whitney *U* test with the Dunn correction were used to compare continuous variables. The Cuzick's non-parametric trend test was used to evaluate the trend across ordinal variables. The correlations between two continuous variables were evaluated using the Spearman test, and the Spearman rank correlation coefficient was represented as r_s. All values are expressed as the median and interquartile range unless otherwise stated. A two-sided *P*-value < 0.05 was considered statistically significant.

Results

Patient characteristics and genotypes

Table 1 shows the demographic and disease characteristics of the enrolled patients. The population consisted of 38 Japanese males and 20 Japanese females. The enrolled patients, whose median age was 76 years, had a high serum level of creatinine (median, 1.67 mg/dL) and low serum level of albumin (median, 3.2 g/dL). The numbers of patients with 3.75, 7.5, 11.25, 15 mg of tolvaptan were 18 (31.0%), 21 (36.2%), 1 (1.7%), and 18 (31.0%), respectively. The median number of days from initiation of tolvaptan therapy was 8 days (interquartile range, 7–11 days) with the maximum of 19 days. The numbers of patients with *CYP3A5*1/*1*, **1/*3*, and **3/*3* were 2 (3.4%), 19 (32.8%), and 37 (63.8%), respectively. The numbers of patients with *ABCB1 3435CC*, *CT*, and *TT* were 18 (31.0%), 27 (46.6%), and 13 (22.4%), respectively. The numbers of patients with *ABCB1 2677GG*, *GT*, *GA*, *TT*, *TA*, and *AA* were 9 (15.5%), 16 (27.6%), 11 (19.0%), 13 (22.4%), 9 (15.5%), and 0 (0%), respectively. The numbers of patients with *ABCB1 1236CC*, *CT*, and *TT* were 11 (19.0%), 27 (46.6%), and 20 (34.5%), respectively. The allele frequencies calculated according to the assumption of the Hardy-Weinberg equilibrium were 80.2%, 45.7%, 61.2%, and 57.8% for the genetic variants of *CYP3A5*3*, *ABCB1 3435C>T*, *2677G>T/A*, and *1236C>T*, respectively. Eleven (19.0%) patients had the ABCB1 triple *TT* diplotype among the 58 enrolled patients. No one had the *3435TT-2677TA-1236TT* or *3435TT-2677AA-1236TT* genotypes in the present study.

Influence of CYP3A5 and ABCB1 genotypes on tolvaptan pharmacokinetics

Table 2 summarizes the plasma exposure parameters of tolvaptan and its metabolites in the heart failure patients with *CYP3A5*1* allele and **3/*3*. The plasma concentration of tolvaptan was significantly higher in the patients with *CYP3A5*3/*3* than with **1* allele ($P = 0.038$). The plasma tolvaptan concentration had a descending trend across the patients with **3/*3*, **1/*3*, and **1/*1* ($P = 0.023$). No significant difference was found in the plasma concentrations of the tolvaptan metabolites and the metabolic ratios of tolvaptan between the CYP3A5 genotypes. Table 3 shows the plasma exposure parameters of tolvaptan and its metabolites in the patients with *ABCB1 3435CC*, *CT*, and *TT*. The plasma tolvaptan pharmacokinetics for *ABCB1 2677G>T/A* and *1236C>T* genotypes were summarized in Table S1 and S2, respectively. No differences were observed in the plasma concentrations of tolvaptan and its metabolites and their metabolic ratios between the ABCB1 genotypes. Between the ABCB1 triple *TT* diplotype and the others, there were no significant differences in the plasma concentrations of tolvaptan and its metabolites (Table S3). The patients with the ABCB1 triple *TT* diplotype had a lower metabolic ratio of DM-4111 to tolvaptan than the others ($P = 0.038$).

Influence of gender on tolvaptan pharmacokinetics

The dose and body weight-adjusted plasma concentration of DM-4103 was higher in male patients than in female ones ($P = 0.030$). No other significant differences were observed in the plasma exposure parameters between genders (Table S4). The medians of the plasma tolvaptan exposure parameters in each gender group between the CYP3A5 genotype and between the

ABCB1 triple *TT* diplotype were summarized in Table S5 and S6, respectively. The patients with the ABCB1 triple *TT* diplotype had a higher absolute plasma concentration of tolvaptan than the others among male patients (P =0.037). The plasma concentration ratios of tolvaptan to DM-4111 (P =0.028) and DM-4103 (P =0.023) were lower in the patients with the ABCB1 triple *TT* diplotype than in the others among male patients, respectively. No other relationships were observed among the genotypes between genders.

Relationship between CYP3A activity markers and CYP3A5 genotype

Table 4 is a summary of the plasma markers of CYP3A activity in the heart failure patients with each CYP3A5 genotype. No differences were observed in the plasma concentrations of 4β-OHC and total cholesterol between the CYP3A5 genotypes. The patients with the *CYP3A5*1* allele did not have a higher plasma 4β-OHC/TC than those with **3/*3* (P = 0.071). The CYP3A5 gene mutation was not associated with plasma 25-OHD concentration. No correlation was found between plasma 4β-OHC/TC and 25-OHD concentration for each CYP3A5 genotype.

Relationship between plasma 4β-OHC and tolvaptan pharmacokinetics

Figure 1 demonstrates the correlations between plasma 4β-OHC/TC and plasma concentrations of tolvaptan and its metabolites in these heart failure patients. Plasma 4β-OHC/TC had no correlation with the plasma concentration of tolvaptan (r_s = -0.049, P = 0.714), DM-4110 (r_s =

-0.070, P = 0.601), DM-4111 (r_s = -0.138, P = 0.317), DM-4119 (r_s = -0.097, P = 0.469), and DM-4103 (r_s = -0.234, P = 0.077) in the whole population. In the patients with the *CYP3A5*1* allele, plasma 4β-OHC/TC showed significant negative correlations with the plasma concentrations of tolvaptan (r_s = -0.446, P = 0.043), DM-4110 (r_s = -0.522, P = 0.015), and DM-4119 (r_s = -0.439, P = 0.047). No association was observed between plasma 4β-OHC/TC and the plasma concentrations of DM-4111 and DM-4103 in the patients with the *CYP3A5*1* allele. In the *CYP3A5*3/*3* patients, plasma 4β-OHC/TC did not associate with the plasma concentrations of tolvaptan and its metabolites. Plasma 4β-OHC/TC had no relationship with the metabolic ratios of tolvaptan. Even in the stratified analysis based on CYP3A5 genotype, no correlation was observed between plasma 4β-OHC/TC and the metabolic ratios of tolvaptan (Figure S2).

Relationship between plasma tolvaptan pharmacokinetics and 25-OHD

The plasma concentration of 25-OHD did not associate with that of tolvaptan (r_s = -0.132, P = 0.324), DM-4110 (r_s = -0.077, P = 0.568), DM-4111 (r_s = -0.064, P = 0.634), DM-4119 (r_s = -0.024, P = 0.860), and DM-4103 (r_s = 0.139, P = 0.299) in the whole population. A negative correlation between the plasma concentrations of 25-OHD and tolvaptan in the patients with *CYP3A5*3/*3* (r_s = -0.284, P = 0.089) did not reach the significance level (Figure S3). No relationship was found between the plasma concentrations of 25-OHD and the tolvaptan metabolites for each CYP3A5 genotype. The plasma concentration of 25-OHD had no

correlations with the metabolic ratios of tolvaptan even in the stratified groups by CYP3A5 genotype. No other associations were observed between the plasma concentration of 25-OHD and the metabolic ratios of tolvaptan for each CYP3A5 genotype (Figure S3).

Factors related to tolvaptan pharmacokinetics

In the multivariate regression analysis, the CYP3A5 genotype was chosen as the explanatory variable for the plasma concentrations of tolvaptan (b = -372.6, P = 0.020), DM-4110 (b = -32.3, P = 0.026), DM-4111 (b = -110.1, P = 0.047), and DM-4119 (b = -16.5, P = 0.042), although no explanatory variable was found for the plasma concentration of DM-4103. The metabolic ratio to DM-4110 (b = 0.041, P = 0.047) was also correlated with the CYP3A5 genotype. Serum albumin level was correlated negatively with the plasma DM-4111 (b = -131.8, P = 0.004) and positively with the metabolic ratio to DM-4103 (b = 11.5, P = 0.036).

Factors related to laboratory test values

No correlation was observed between the absolute plasma tolvaptan concentration and the major laboratory test values (Figure 2). The absolute plasma concentration of tolvaptan also had no association with the serum levels of chloride (r_s = -0.197, P = 0.138), aspartate aminotransferase (r_s = 0.054, P = 0.685), and alanine aminotransferase (r_s = -0.112, P = 0.401). With respect to the tolvaptan metabolites, the absolute plasma concentration of DM-4111 was negatively correlated with the serum albumin level (r_s = -0.286, P = 0.029) (Figure S4). The

patients with *CYP3A5*3/*3* had a higher serum sodium level than those with **1* allele (P = 0.038). There were no differences in the other laboratory test values between the CYP3A5 genotypes. Plasma 4β-OHC/TC (r_s = -0.245, P = 0.064) and 25-OHD concentration (r_s = 0.149, P = 0.266) had no correlation with serum sodium level. The plasma 25-OHD concentration showed a significant positive correlation with serum total bilirubin level (r_s = 0.335, P = 0.010), while plasma 4β-OHC/TC did not (r_s = -0.086, P = 0.521). The CYP3A activity markers did not associate with the other laboratory test values. The ABCB1 genotype had no relation to the laboratory test values.

Discussion

The present study investigated the influence of CYP3A5 and ABCB1 genotypes on tolvaptan pharmacokinetics and their relationships with plasma markers of CYP3A activity and laboratory test values in 58 heart failure patients. The CYP3A5 gene mutation elevated the plasma tolvaptan concentration, while the ABCB1 gene mutation did not. Plasma 4β-OHC/TC had no association with the plasma tolvaptan concentration in the whole population. In the *CYP3A5*1* allele carriers, the plasma 4β-OHC/TC showed a negative correlation with plasma tolvaptan concentration. In addition, the tolvaptan-treated volume overloaded patients lacking CYP3A5 had a higher serum sodium level. These findings suggest that the CYP3A5 genotype affects not only plasma tolvaptan and its relationship with plasma 4β-OHC but also serum sodium. To the best of our knowledge, this is the first report that has characterized the genetic

impact on tolvaptan pharmacokinetics and laboratory test values using endogenous markers of CYP3A activity in heart failure patients.

The patients with homozygous *CYP3A5*3* allele had an approximately 2-fold higher plasma concentration of tolvaptan than those with **1* allele. Tolvaptan was predominantly metabolized by CYP3A4/5 using recombinant CYP isoforms [7]. The respective contributions of CYP3A4 and 3A5 to tolvaptan plasma exposure have not been clarified in humans. CYP3A5-deficient patients had close to double the plasma concentrations of some CYP3A5 substrates [12,13]. The CYP3A5 genotype had no relationship with the metabolic ratios of tolvaptan in the present study. These data indicate that CYP3A5 participates in both the production and elimination of the tolvaptan metabolites [7]. In the monohydroxylation of tolvaptan, the metabolic ratio to DM-4111 was much higher than that to DM-4110 and DM-4119. The pathway from tolvaptan to DM-4111, which is potentially influenced by serum albumin level, seems to be comparatively dominant in heart failure patients.

The ABCB1 genotype had no relationship with the plasma concentrations of tolvaptan and its metabolites although the patients with the ABCB1 triple *TT* diplotype had a lower metabolic ratio to DM-4111 than the others. These results imply that the ABCB1 genotype does not strongly alter the intestinal absorption of tolvaptan in heart failure patients. Digoxin, an ABCB1 inhibitor, slightly increased the peak plasma concentration of tolvaptan in healthy subjects [10]. Rheumatoid arthritis patients with *ABCB1 3435TT* had a 50% higher blood concentration of tacrolimus than those with *3435CC* [15]. The absolute bioavailability of oral

tolvaptan was 56% with an interquartile range of 42–80% in healthy subjects [2]. Other mechanisms such as the intestinal metabolism and transport system may be responsible for interindividual variations in the oral bioavailability of tolvaptan [26].

Plasma 4β-OHC and 4β-OHC/TC were 1.4- and 1.6-fold higher, respectively, in the patients with the *CYP3A5*1* allele than in those with **3/*3*, although the differences were not significant. Higher plasma 4β-OHC was observed in kidney transplant recipients with the *CYP3A5*1* allele [27]. The *CYP3A5*1* allele carriers had 1.5-fold higher plasma 4β-OHC and 1.7-fold higher plasma 4β-OHC/TC in cancer patients [13]. In the present study population, plasma 4β-OHC may not be useful for clear discrimination of the homozygous *CYP3A5*3* allele. The plasma 25-OHD level was not different between the CYP3A5 genotypes. Plasma 25-OHD, an indicator of nutritional intake and vitamin D synthesis, has seasonal variation caused by sunlight exposure [28,29]. Our data support that plasma 25-OHD is independent of the CYP3A5 genotype [30].

Plasma 4β-OHC/TC had a negative correlation with plasma tolvaptan in the *CYP3A5*1* allele carriers, despite no correlation in the whole population. Plasma 4β-OHC/TC can be useful to predict tolvaptan pharmacokinetics in *CYP3A5*1* allele carriers. Both CYP3A4 and 3A5 mediate the cholesterol biotransformation to 4β-OHC [17]. *In vitro* metabolism of tolvaptan was faster using human liver microsomes with *CYP3A5*1/*1* than with **1/*3* or **3/*3* [7]. Our data indicate that tolvaptan is metabolized by CYP3A5 rather than CYP3A4 in humans. In the *CYP3A5*1* allele carriers, plasma 4β-OHC/TC had a negative correlation with

the plasma concentrations of DM-4110 and DM-4119, which may be also principally eliminated by CYP3A5. In contrast, the plasma concentration of DM-4103, a ring-opened metabolite produced via a three-step oxidation of tolvaptan (Figure S1), had no relationship with plasma 4β-OHC/TC in the *CYP3A5*1* allele carriers. Plasma 4β-OHC/TC did not fully reflect the multi-step metabolism from tolvaptan to DM-4103.

Plasma 25-OHD had no relevance to plasma tolvaptan in the whole population. A negative correlation was not significant between plasma 25-OHD and tolvaptan even in the patients lacking CYP3A5. Vitamin D increases expression of intestinal CYP3A4 rather than hepatic CYP3A4 [19]. The present results suggest that intestinal CYP3A4 does not participate in tolvaptan metabolism. However, the peak plasma concentration of tolvaptan increased twice with a single co-administration of grapefruit juice, probably due to inhibition of intestinal CYP3A4 [26]. Observation of the peak plasma concentration of tolvaptan would confirm the usefulness of plasma 25-OHD for the prediction of tolvaptan pharmacokinetics.

The CYP3A5-deficient volume overloaded patients had a higher serum sodium level in this study. Tolvaptan potentially causes severe hypernatremia at therapeutic doses [5]. Our data revealed that the *CYP3A5*3* allele raised the plasma tolvaptan concentration. The effect of tolvaptan on serum sodium usually appeared within five days after starting the medication [31]. The point of blood sampling seems appropriate to evaluate serum levels of electrolytes in the present study. However, the absolute plasma concentrations of tolvaptan and its metabolites had no relation to the serum sodium level. Careful monitoring of the serum sodium level likely leads

to the modification of the prescription behavior of other diuretics by physicians in clinical settings. Further investigations, including sodium and water intake, and co-treatment with other diuretics may elucidate the impact of plasma tolvaptan on serum sodium levels.

The absolute plasma concentration of DM-4103 exceeded 122 ng/mL, the 50% inhibitory concentration for OATP1B1, in most of the enrolled patients. The 50% inhibitory concentration of DM-4103 is > 1.9 μg/mL for other bile acid transporters such as bile salt export pump and P-glycoprotein [8]. DM-4103 is assumed to be the most relevant to tolvaptan-induced liver injury among tolvaptan and its metabolites [9]. However, the serum liver function test results did not associate with the absolute plasma concentration of DM-4103 in this study. A slight elevation of serum total bilirubin caused by plasma DM-4103 exposure may not be detectable by observation at a single point in the short term after starting tolvaptan treatment. Investigation into longitudinal change of the total bilirubin level and genetic impairment of OATP1B1 would reveal the relationship between absolute plasma DM-4103 and serum bilirubin [32].

The serum total bilirubin level had a positive correlation with plasma 25-OHD in the present study. A decreased serum level of total bilirubin, which has an antioxidant effect, was a potential risk factor for renal dysfunction [33,34]. Plasma 25-OHD positively correlated with the glomerular filtration rate in patients with chronic kidney disease [35,36]. In our study population, the serum total bilirubin level did not have a significant positive correlation with the estimated glomerular filtration rate (r_s = 0.226, P = 0.088) and plasma 25-OHD (r_s = 0.187,

P = 0.160). Confounding factors, which might include renal function, intermediate between serum total bilirubin and plasma 25-OHD.

A few limitations need to be addressed. First, the present study did not include patients with liver cirrhosis or with serum total bilirubin > 2.0 mg/dL before starting tolvaptan treatment. CYP3A activity was impaired in liver cirrhosis patients with Child-Pugh class B or C [37]. Tolvaptan was predominantly eliminated by hepatic CYP3A4/5 [7]. Careful consideration is required to apply our findings to liver cirrhosis patients. Second, the usefulness of plasma 4β-OHC or 4β-OHC/TC as a marker of CYP3A activity is controversial in some recent literatures. The specificity of 4β-OHC has been criticized for the lack of verification on its formation and clearance [38]. In contrast, Gravel *et al*. suggested that 4β-OHC is considered as a suitable endogenous biomarker to evaluate in CYP3A activity based on the strong correlation of plasma 4β-OHC and 4β-OHC/TC with midazolam metabolic ratio observed in patients [39]. Third, only one blood sample was collected for each patient in this study. The predose plasma concentration of tolvaptan was found to be highly correlated with its plasma exposure [6]. The employment of a single sampling point can result in uncertainty in the pharmacokinetic evaluation. Further analysis with a plasma concentration profile of tolvaptan would corroborate our findings.

The clinical implications for volume overload management using tolvaptan have not been fully clarified in this study. However, the patients with the *CYP3A5*1* allele and high plasma 4β-OHC, who had a lower plasma concentration of tolvaptan, may need a higher dose

of tolvaptan in order to obtain adequate efficacy. In contrast, the incidence of hypernatremia is assumed to be higher in patients with *CYP3A5*3/*3* or low plasma 4β-OHC. More clinical data are required to apply our findings to the dose optimization of tolvaptan in heart failure patients.

In conclusion, *CYP3A5*3* but not *ABCB1 3435C>T* elevated tolvaptan plasma exposure in heart failure patients. CYP3A5-deficient patients treated with tolvaptan had a higher serum sodium level. The CYP3A5 genotype altered the relationship between plasma tolvaptan and 4β-OHC.

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Table 1. Patient characteristics in this study population

(A) Demographic characteristics	
Gender, male/female	38/20
Age, years	76 (70–82)
Body weight, kg	53.9 (47.8–64.5)
Serum sodium, mEq/L	141 (139–143)
Serum potassium, mEq/L	4.4 (3.9–4.8)
Serum chloride, mEq/L	104 (100–106)
Serum total protein, g/dL	6.2 (5.7–6.7)
Serum albumin, g/dL	3.2 (2.6–3.4)
Serum creatinine, mg/dL	1.67 (1.27–2.31)
Estimated glomerular filtration rate, mL/min	25.7 (17.7–36.9)
Serum blood urea nitrogen, mg/dL	33.8 (24.2–44.4)
Serum total bilirubin, mg/dL	0.6 (0.5–0.9)
Serum aspartate aminotransferase, IU/L	22 (17–32)
Serum alanine aminotransferase, IU/L	15 (10–27)
Serum C-reactive protein, mg/dL	0.97 (0.31–2.94)

(B) Disease characteristics	
Acute/chronic heart failure	20/38
NYHA classification, II/III/IV	28/19/11
Underlying heart disease	
Ischemic heart disease	20
Valvular heart disease	25
Disease complication	
Atrial fibrillation	19
Diabetes mellitus	27
Hypertension	25
Concomitant diuretic category	
Loop diuretic alone	38
Loop diuretic + aldosterone antagonist	12
Loop diuretic + thiazide diuretic	7
None	1

Data are expressed as the number of patients or median with interquartile range in parentheses.
NYHA, New York Heart Association

Table 2. Influence of CYP3A5 genotype on plasma exposure parameters of tolvaptan and its metabolites in heart failure patients

CYP3A5 genotype	**1* allele carriers, $n = 21$	**3/*3*, $n = 37$	*P* value
Absolute plasma tolvaptan, ng/mL	22.1 (13.9–38.7)	29.1 (20.5–77.2)	0.069
Absolute plasma DM-4110, ng/mL	3.27 (2.41–4.93)	4.52 (2.65–9.81)	0.174
Absolute plasma DM-4111, ng/mL	22.9 (11.7–37.8)	30.1 (16.9–60.0)	0.143
Absolute plasma DM-4119, ng/mL	2.75 (1.97–4.36)	4.01 (2.29–7.77)	0.064
Absolute plasma DM-4103, ng/mL	204 (125–337)	380 (163–1123)	0.053
Plasma tolvaptan, ng/mL per mg/kg	165 (93–286)	304 (147–940)	0.038
Plasma DM-4110, ng/mL per mg/kg	29.2 (14.8–45.5)	33.9 (19.7–104.8)	0.172
Plasma DM-4111, ng/mL per mg/kg	173 (126–225)	206 (121–369)	0.304
Plasma DM-4119, ng/mL per mg/kg	21.9 (14.5–30.7)	30.9 (20.1–47.7)	0.057
Plasma DM-4103, ng/mL per mg/kg	1879 (1061–3017)	2417 (1606–6082)	0.066
Plasma concentration ratio of DM-4110 to tolvaptan	0.119 (0.094–0.247)	0.130 (0.094–0.147)	0.261
Plasma concentration ratio of DM-4111 to tolvaptan	1.10 (0.40–1.44)	0.710 (0.545–0.981)	0.289
Plasma concentration ratio of DM-4119 to tolvaptan	0.132 (0.071–0.227)	0.0989 (0.0762–0.1378)	0.247
Plasma concentration ratio of DM-4103 to tolvaptan	14.8 (2.8–29.1)	9.08 (4.51–21.68)	0.790

Data are expressed as median with interquartile range in parentheses. All statistics were analyzed using the Mann-Whitney *U* test.

Table 3. Influence of *ABCB1 3435C>T* genotype on plasma exposure parameters of tolvaptan and its metabolites in heart failure patients

ABCB1 3435C>T genotype	*3435CC*, *n* = 18	*3435CT*, *n* = 27	*3435TT*, *n* = 13	*P* value
Absolute plasma tolvaptan, ng/mL	22.3 (12.4–39.5)	29.1 (19.1–108.6)	28.6 (22.7–68.4)	0.379
Absolute plasma DM-4110, ng/mL	2.70 (1.99–4.70)	5.83 (2.62–10.43)	4.70 (3.08–8.03)	0.090
Absolute plasma DM-4111, ng/mL	18.4 (10.3–38.3)	31.6 (16.7–66.0)	20.0 (16.9–37.8)	0.332
Absolute plasma DM-4119, ng/mL	2.33 (1.92–3.39)	4.14 (2.31–7.65)	4.32 (2.60–5.78)	0.082
Absolute plasma DM-4103, ng/mL	339 (150–826)	259 (141–1123)	380 (160–885)	0.974
Plasma tolvaptan, ng/mL per mg/kg	197 (98–430)	205 (104–637)	323 (147–586)	0.697
Plasma DM-4110, ng/mL per mg/kg	27.0 (17.5–39.1)	36.7 (17.7–63.4)	33.4 (21.0–55.0)	0.429
Plasma DM-4111, ng/mL per mg/kg	174 (112–416)	248 (126–344)	161 (126–248)	0.413
Plasma DM-4119, ng/mL per mg/kg	21.0 (16.1–32.8)	30.4 (21.0–50.2)	29.9 (20.1–34.2)	0.420
Plasma DM-4103, ng/mL per mg/kg	2338 (1721–6139)	1922 (1161–4546)	1976 (1363–5132)	0.626
Plasma concentration ratio of DM-4110 to tolvaptan	0.137 (0.105–0.155)	0.113 (0.088–0.230)	0.129 (0.103–0.139)	0.882
Plasma concentration ratio of DM-4111 to tolvaptan	0.810 (0.404–1.140)	0.740 (0.545–1.603)	0.622 (0.284–1.081)	0.552
Plasma concentration ratio of DM-4119 to tolvaptan	0.0987 (0.0722–0.1898)	0.107 (0.053–0.185)	0.104 (0.074–0.133)	0.981
Plasma concentration ratio of DM-4103 to tolvaptan	14.4 (4.7–37.4)	9.08 (3.81–23.77)	7.04 (2.34–16.64)	0.502

Data are expressed as median with interquartile range in parentheses. All statistics were analyzed using the Kruskal-Wallis test.

Table 4. Relationship between CYP3A activity markers and CYP3A5 genotype in heart failure patients

CYP3A5 genotype	**1* allele carriers, $n = 21$	**3/*3*, $n = 37$	P value
Plasma 4β-OHC, ng/mL	70.5 (52.2–91.1)	49.4 (34.0–109.5)	0.132
Plasma total cholesterol, mg/dL	132 (120–173)	154 (141–177)	0.213
Plasma 4β-OHC/TC, $\times 10^{-6}$	50.2 (40.1–77.9)	32.1 (21.2–65.6)	0.071
Plasma 25-OHD, ng/mL	40.2 (22.6–46.1)	42.4 (32.3–58.9)	0.126

Data are expressed as median with interquartile range in parentheses. All statistics were analyzed using the Mann-Whitney *U* test.

4β-OHC, 4β-hydroxycholesterol; 4β-OHC/TC, 4β-hydroxycholesterol/total cholesterol ratio; and 25-OHD, 25-hydroxyvitamin D.

Figure 1

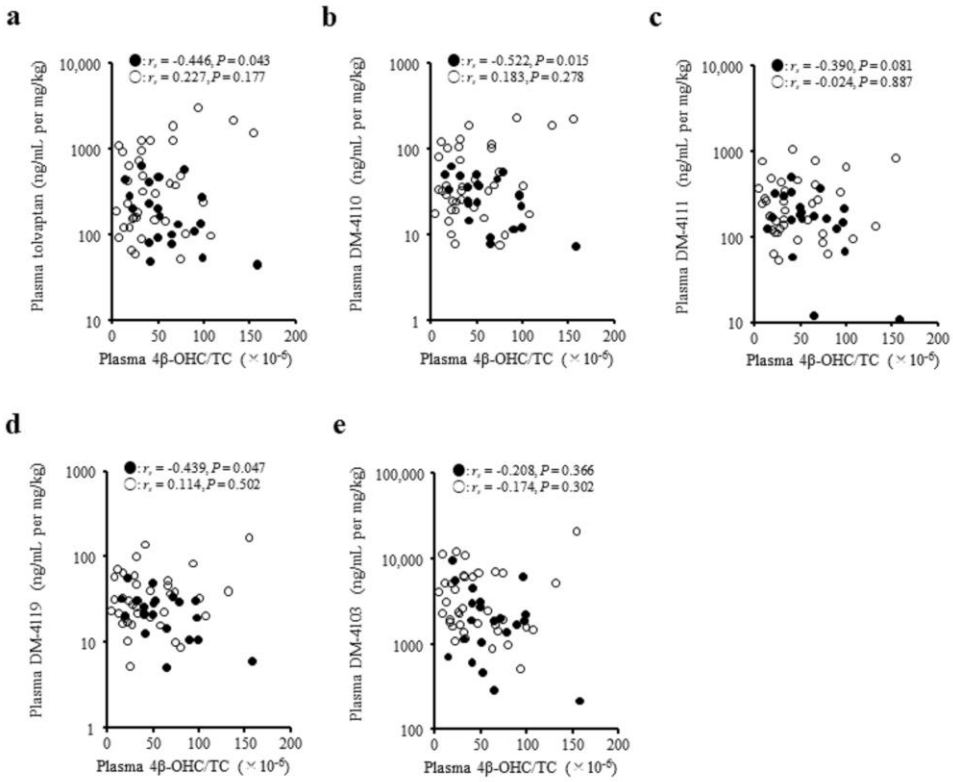

Figure 1. Correlations between plasma 4β-hydroxycholesterol/total cholesterol ratio (4β-OHC/TC) and plasma concentrations of tolvaptan and its metabolites in heart failure patients.

Plasma 4β-OHC/TC versus (a) tolvaptan, (b) DM-4110, (c) DM-4111, (d) DM-4119, and (e) DM-4103. Solid circles: patients with the *CYP3A5*1* allele, open circles: patients with the *CYP3A5*3/*3* allele. The correlations were evaluated using the Spearman test.

Figure 2

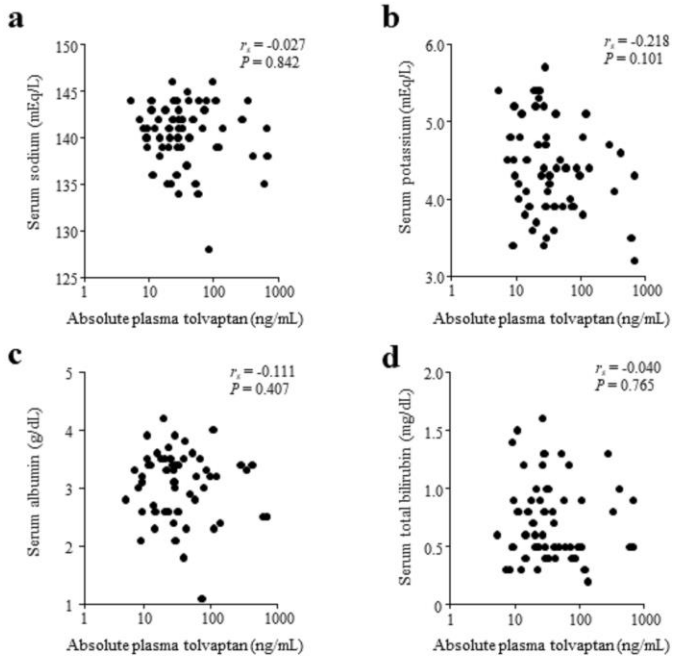

Figure 2. Correlations between absolute plasma tolvaptan concentration and laboratory test values in heart failure patients.

(a) Serum sodium, (b) potassium, (c) albumin, and (d) total bilirubin. The correlations were evaluated using the Spearman test.