Nonlinear Mixed Effects Model Analysis of the Pharmacokinetics of Metoprolol in Routinely Treated Japanese Patients

Masato TAGUCHI,^a Takashi NOZAWA,^b Kouichi MIZUMAKI,^b Hiroshi INOUE,^b Katsutoshi TAHARA,^a Chihiro TAKESONO,^a and Yukiya HASHIMOTO^{*,a}

^a Graduate School of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University; and ^b Second Department of Internal Medicine, Faculty of Medicine, Toyama Medical and Pharmaceutical University; 2630 Sugitani, Toyama 930-0194, Japan. Received April 5, 2004; accepted July 23, 2004

This study was performed to estimate the mean pharmacokinetic parameters of routinely administered metoprolol in middle-aged and elderly Japanese patients. Whole blood concentration data (65 samples) at steady-state following repetitive administration to 34 patients were analyzed using a nonlinear mixed effects model. A one-compartment model was parameterized in terms of oral clearance (CL/F) and apparent volume of distribution (V/F). We evaluated the effect of polymorphic alleles ($CYP2D6^*2$, $CYP2D6^*10$, $CYP2C19^*2$ and $CYP2C19^*3$), age, gender, and heart failure on the pharmacokinetic parameters of metoprolol. The CL/F value in patients homozygous for the $CYP2D6^*10$ allele was 64% lower than that in patients with a $CYP2D6^*1^*1$ or $*1^*2$ genotype. The CL/F value in older (>70 years old) patients was 26% lower than that in younger (≤ 70 years old) patients. In addition, the V/F value in patients homozygous for the $CYP2D6^*10$ allele was 25% lower than that in patients with the $CYP2D6^*1^*1$ or $*1^*2$ genotype. On the other hand, the $CYP2C19$ genotype, gender, and heart failure showed no significant effects on the pharmacokinetics of metoprolol. The results suggest that the pharmacokinetic variability of metoprolol in Japanese extensive metabolizers of $CYP2D6$ is very large, probably because $CYP2D6^*10$ is responsible not only for the decreased systemic clearance (CL) but also for the increased bioavailability (F) of the drug.

Key words aging; Japanese patient; metoprolol; nonlinear mixed effects model (NONMEM); pharmacokinetics; whole blood concentration

$CYP2D6$ genetic polymorphisms have been extensively studied, and more than 40 variants have been documented.¹⁾ In addition, a pronounced interethnic difference is present in the allele frequencies of individual variants of $CYP2D6$. Poor metabolism due to $CYP2D6$ occurs in 7% of Caucasians but less than 1% of Asians.^{2,3)} Among Asian extensive metabolizers, the three most common alleles of the $CYP2D6$ gene are $CYP2D6^*1$, $CYP2D6^*2$, and $CYP2D6^*10$.⁴⁾ $CYP2D6^*1$ produces a wild-type protein with Pro³⁴, Arg²⁹⁶, and Ser⁴⁸⁶. $CYP2D6^*2$ produces a mutant enzyme with Arg²⁹⁶Cys and Ser⁴⁸⁶Thr amino acid substitutions,⁵⁾ and $CYP2D6^*10$ produces an enzyme with Pro³⁴Ser and Ser⁴⁸⁶Thr amino acid substitutions.^{6,7)} The allele frequencies of $CYP2D6^*1$, $*2$, and $*10$ in Asian subjects are 23–45%, 9–20%, and 38–54%, respectively, whereas the allele frequencies of $CYP2D6^*1$, $*2$, and $*10$ in Caucasian subjects are 33–40%, 22–34%, and 1–8%, respectively.^{1,8–10)} Recently, we have examined the effect of $CYP2D6^*2$ and $CYP2D6^*10$ on the steady-state plasma concentration of routinely administered metoprolol in middle-aged and elderly Japanese patients, and found that $CYP2D6^*10$ but not $CYP2D6^*2$ is largely responsible for the pharmacokinetic variability of metoprolol.¹¹⁾

Metoprolol is extensively metabolized in the liver. Therefore, the orally administered metoprolol is subjected to avid hepatic first-pass extraction.^{12,13)} Wilkinson and Shand have stated as follows: “since drug distributed in the erythrocyte usually equilibrates rapidly with that in the plasma, and any drug in the red blood cells is generally available for hepatic extraction, and blood rather than plasma concentrations must be used when any physiological interpretation is placed on the clearance process.”¹⁴⁾ Previously, we have reported that the V/F value of metoprolol in Japanese patients with $CYP2D6^*10$ is lower than that in patients without

$CYP2D6^*10$, and suggested that hepatic first-pass extraction may be decreased in patients with $CYP2D6^*10$.¹¹⁾ Therefore, in this study, we analyzed newly measured blood concentrations of metoprolol to evaluate hepatic first-pass extraction and bioavailability (F) of the drug.

The protein binding of metoprolol in human plasma is only about 12%.¹³⁾ Therefore, it is unlikely that the alteration of plasma unbound fraction of the drug is responsible for the pharmacokinetic variability in patients. On the other hand, several studies reported on the effect of age on the pharmacokinetics of metoprolol. Regårdh *et al.* found that there is no effect of age on the pharmacokinetics of metoprolol.¹⁵⁾ In contrast, Kendall *et al.* reported that the mean plasma levels of metoprolol tend to be higher in elderly normotensive subjects (62–77 years old) than in middle-aged hypertensive patients (50–61 years old) and young healthy volunteers (20–26 years old).¹⁶⁾ Rendic suggested that not only $CYP2D6$ but also $CYP2C19$ contributes to the metabolism of metoprolol as well as propranolol.¹⁷⁾ In addition, Luzier *et al.* reported that the oral clearance of metoprolol in young white women is significantly lower than that in young white men.¹⁸⁾ However, the effects of aging and gender on the pharmacokinetics or the contribution of $CYP2C19$ to the metabolism of metoprolol is unclear for the Japanese population. Accordingly, this study was designed to investigate the effect of these factors on the oral clearance (CL/F) and apparent volume of distribution (V/F) of metoprolol, where a pharmacokinetic analysis was performed using a nonlinear mixed effects model (NONMEM) program.

MATERIALS AND METHODS

Subjects and Study Design Thirty-four subjects in this

* To whom correspondence should be addressed. e-mail: yukiya@ms.toyama-mpu.ac.jp

study were the same one who participated in a previous study on plasma metoprolol concentration, and their characteristics have been previously described.¹¹⁾ In brief, the subjects were Japanese patients consisting of 21 males and 13 females between 56 and 83 (mean $\pm$ S.D.: 69.6 $\pm$ 7.6) years old, and their mean body weight ($\pm$ S.D.) was 60.0 $\pm$ 11.0 kg. No patients had null alleles of *CYP2D6* (*4, *5, *14), and they could be divided into five groups on the basis of *CYP2D6* genotypes.¹¹⁾ Nine patients were homozygous for the *CYP2D6**1 allele (Group 1a), and five were heterozygous for the *CYP2D6**1/*2 alleles (Group 1b). Finally, seven patients were heterozygous for the *CYP2D6**1/*10 alleles (Group 2a), and six patients were heterozygous for the *CYP2D6**2/*10 alleles (Group 2b). Seven patients were homozygous for the *CYP2D6**10 allele (Group 3). In this study, five patients had symptomatic congestive heart failure (CHF): four patients were characterized as New York Heart Association (NYHA) class II, and one patient was NYHA class III. However, no patients had severe hepatic or renal failure, and none had received any potent inhibitor of *CYP2D6* (e.g. amiodarone or quinidine). These patients had been routinely treated with oral administration of a rapid-release preparation of metoprolol tartrate (Seloken® Tablets, AstraZeneca, Tokyo, Japan) at doses between 40 and 120 mg/d, and the drug was administered twice a day in 8 patients and three times a day in 26 patients. The peak blood concentration occurs within 2 h after dosing of a rapid-release preparation of metoprolol tartrate.¹⁹⁾ Therefore, blood samples at steady-state following repetitive administration were obtained between 2 and 4.5 h after dosing for all 34 patients. Additional blood samples just before dosing were obtained from 31 patients. All patients gave written consent to participate in this study, which was approved by the ethics committee of Toyama Medical and Pharmaceutical University.

Genotyping of CYP2C19 Genomic DNA in 34 patients was isolated from peripheral blood, and was stored at -80°C .¹¹⁾ The *CYP2C19**1 (wild-type) allele and two defective allelic variants, *CYP2C19**2 and *CYP2C19**3, were determined using the polymerase chain reaction-restriction fragment length polymorphism method as described by Lamba *et al.*²⁰⁾ The homozygotes of *CYP2C19**1 and the heterozygotes of mutant alleles (*CYP2C19**1/*2 and *1/*3) were defined as extensive metabolizers of *CYP2C19*. The homozygotes for mutant alleles (*CYP2C19**2/*2, *2/*3, and *3/*3) were defined as poor metabolizers of *CYP2C19*.

Assay of Metoprolol in Whole Blood The blood metoprolol concentration was measured using HPLC as described by Hamelin *et al.* with minor modifications.²¹⁾ Briefly, whole blood samples (0.5 ml) were mixed with an identical volume of distilled water to hemolyze the blood cells. After alkalization with 3 ml of glycine buffer (0.1 M, saturated with NaCl, pH 10.6), the samples were mildly extracted with 5 ml of diethylether for 20 min. Metoprolol was back-extracted from the organic phase with 0.2 ml of 0.05 N HCl. A 50 μl aliquot of HCl solution was injected onto the HPLC system. The column was a COSMOSIL 5C18-AR-II (15 cm $\times$ 4.6 mm; i.d. 4.5 μm particle size; Nacalai tesque). The mobile phase consisted of 10 mM KH_2PO_4 /acetonitrile (90.5/9.5) that contained 0.6% (wt/vol) triethylamine adjusted to pH 3.3 with phosphoric acid. The peaks were monitored at an exci-

tation wavelength of 272 nm and an emission wavelength of 303 nm. The coefficient of variation for this assay was 2.1% and 1.3% at whole blood concentrations of 5 ng/ml and 50 ng/ml, respectively. The detection limit for metoprolol was 2 ng/ml for its whole blood concentration.

Nonlinear Mixed Effects Model of the Pharmacokinetics of Metoprolol The time required to reach the peak concentration after oral administration of a rapid-release preparation of metoprolol (e.g. Seloken® Tablets) is known to be short.¹⁹⁾ Therefore, the one-compartment model with repetitive bolus dosing was parameterized in terms of CL/F and V/F .²²⁾ For the simplest basic model (Model 1), the oral clearance in the i th individual (CL/F_i) was modeled using the following equation:

$$CL/F_i = \theta_1 \cdot WT_i \cdot (1 + \eta_{CL/F_i}) \quad (1)$$

where θ_1 is the predicted population mean of the oral clearance, WT_i is the individual body weight, and η_{CL/F_i} is a random variable distributed with a mean of zero and variance of $\omega_{CL/F}^2$. The apparent volume of distribution in the i th individual (V/F_i) was modeled using the following equation:

$$V/F_i = \theta_2 \cdot WT_i \cdot (1 + \eta_{V/F_i}) \quad (2)$$

where θ_2 is the predicted population mean of the apparent distribution volume, and η_{V/F_i} is a random variable with a mean of zero and variance of $\omega_{V/F}^2$. Finally, the j th observed blood concentration in the i th patient (Ch_{ij}) was assumed to be randomly and normally distributed from the j th predicted blood concentration in the i th patient (Cb_{ij}^*):

$$Ch_{ij} = Cb_{ij}^* + \varepsilon_{ij} \quad (3)$$

where ε_{ij} is a random variable that describes intraindividual variability with a mean of zero and variance of σ^2 .

Table 1 summarizes the 16 analysis models used in this study. The effects of body weight (WT) on CL/F and V/F were evaluated by Model 2 and Model 3, respectively. The effects of the *CYP2D6**10 genotype on CL/F and V/F were evaluated by Models 4, 5, 6, and 7; where $G1=1$ and $G3=0$ for the Group 1 patients with *CYP2D6**1/*1 and *1/*2, $G1=0$ and $G3=0$ for the Group 2 patients with *CYP2D6**1/*10 and *2/*10, and $G1=0$ and $G3=1$ for the Group 3 patients with *CYP2D6**10/*10. The effects of age (AGE) on CL/F and V/F were evaluated by Models 8, 9, and 10; where $AGE > 70$ is one for the older (71–83 years old) patients, and zero for the younger (56–70 years old) patients. The effects of gender on CL/F and V/F were evaluated by Models 11 and 12, respectively; where SEX is one for females and zero for males. The effects of heart failure on CL/F and V/F were evaluated by Models 13 and 14, respectively; where CHF is one for patients with NYHA II-III and zero for patients without heart failure. In addition, the effect of the *CYP2D6**2 genotype on CL/F was evaluated by Model 15; where $2D6*2$ is one for patients with *CYP2D6**2 and zero for patients without *CYP2D6**2. The effect of poor metabolizer genotypes of *CYP2C19* on CL/F was evaluated by Model 16; where $2C19PM$ is one for poor metabolizers of *CYP2C19* and zero for extensive metabolizers of *CYP2C19*.

Data Analysis Data analysis was performed with NONMEM software (double precision NONMEM Version V Level 1.1, PREDPP Version IV Level 1.1, and NM-TRAN Version III Level 1.1)²³⁾ running on a mainframe UNIX com-

puter at the Kyoto University Data Processing Center. In the present study, we used a first-order estimation method and the NONMEM-PREDPP library subroutines ADVAN1 and TRANS2 for the one-compartment model with bolus dosing.²³⁾ Statistical significance of the parameters was evaluated by the likelihood ratio test using the minimum value of the objective function ($-2 \log$ likelihood) produced by NONMEM.²³⁾ That is, when the difference of $-2 \log$ likelihood (LLD) between two models allowing a parameter of interest freely estimated *versus* a fixed hypothetical value was greater than 3.84, the parameter value was considered to be statistically significant ($p < 0.05$). In addition, NONMEM provides estimates of the standard error (S.E.) for all the parameters, and S.E. can be used to define 95% confidence intervals (CI) for true parameter values: 95% CI = (the estimated parameter value) $\pm 1.96 \cdot \text{S.E.}$ ²³⁾

RESULTS

Figure 1 shows the relationship between the measured blood concentration and plasma concentration of metoprolol in 34 Japanese patients. The large interindividual variability was observed in the blood/plasma concentration (B/P) ratio. Figure 2 shows the observed blood concentrations of metoprolol in the 34 patients. The blood metoprolol concentrations of Group 1a (patients with *CYP2D6**1/*1) were similar to those of Group 1b (patients with *CYP2D6**1/*2). There was little difference between the blood concentrations of Group 2a (patients with *CYP2D6**1/*10) and those of Group 2b (patients with *CYP2D6**2/*10), and higher levels of metoprolol were observed in Group 2 (Group 2a plus 2b) compared with Group 1 (Group 1a plus 1b). The blood levels of Group 3 (patients with *CYP2D6**10/*10) were considerably higher than those of Groups 1 and 2.

Sixty-five blood metoprolol concentration data points from 34 patients were analyzed using 16 pharmacokinetic models, and the LLD values are summarized in Table 1. The population mean pharmacokinetic parameters, θ_1 and θ_2 , for Model 1 were estimated to be 0.811 l/h/kg and 5.17 l/kg, respectively. The $\omega_{CL/F}$ and $\omega_{V/F}$ values for Model 1 were estimated to be 69.7% and 38.6%, respectively. The θ_3 value for Model 2 and the θ_4 value for Model 3 were estimated to be 0.722 and 1.15, respectively. However, the LLD values for Models 2 and 3 did not reach a statistically significant level (Table 1).

The θ_5 and $\omega_{CL/F}$ values for Model 4 were estimated to be 0.377 l/h/kg and 36.3%, respectively, and the LLD value was 54.35 ($p < 0.001$) (Table 1), indicating a significant effect of the *CYP2D6**10 allele on the pharmacokinetic variability of metoprolol. However, no further significant improvement by the introduction of θ_6 was observed in the LLD value for Model 5 (Table 1). The θ_7 and $\omega_{V/F}$ values for Model 6 were estimated to be 0.738 l/kg and 13.2%, respectively, and the LLD value was 6.36 ($p < 0.025$). No further improvement by the introduction of θ_8 was observed in the LLD value for Model 7 (Table 1).

The θ_9 value was estimated to be 0.0133 for Model 8; however, the LLD value did not reach a statistically significant level (Table 1). In contrast, the θ_{10} value for Model 9 was estimated to be 0.739, and the LLD value was 6.76 ($p < 0.01$), which indicated that the CL/F value in the older (>70 years old) patients was significantly lower than that in

Fig. 1. Relationship between the Measured Blood Concentration and the Plasma Concentration of Metoprolol in 34 Japanese Patients

Fig. 2. Whole Blood Concentrations of Metoprolol at the Steady-State in Groups 1, 2, and 3

Open circles: *CYP2D6**1/*1 (Group 1a), *CYP2D6**1/*10 (Group 2a), or *CYP2D6**10/*10 (Group 3); solid circles: *CYP2D6**1/*2 (Group 1b) or *CYP2D6**2/*10 (Group 2b).

the younger (≤ 70 years old) patients. The θ_{11} value for Model 10 was estimated to be 0.888, and the LLD value in Model 10 was 1.13. Thus no significant effect of aging on V/F was detected for Model 10 (Table 1).

The θ_{12} value for Model 11 and the θ_{13} value for Model 12 were estimated to be 0.848 and 0.951, respectively. The θ_{14} value for Model 13 and the θ_{15} value for Model 14 were estimated to be 0.863 and 1.04, respectively. The θ_{16} value for Model 15 and the θ_{17} value for Model 16 were estimated to be 1.19 and 0.974, respectively. However, the LLD values in Models 11–16 did not reach a statistically significant level (Table 1). Accordingly, Model 9 was selected as the final model to describe the pharmacokinetics of routinely administered metoprolol in middle-aged and elderly Japanese patients.

Table 2 shows the final estimates of population pharmacokinetic parameters of metoprolol and their 95% CI for Model 9. The final CL/F (θ_1) and V/F (θ_2) values in Group 2 were estimated to be 0.938 l/h/kg and 4.52 l/kg, respectively. On the other hand, the CL/F value in Group 1 ($\theta_1 + \theta_5$) was 2.76 times as large as that in Group 3 ($\theta_1 - \theta_5$). In addition, the mean CL/F value in the older (>70 years old) patients was

Table 1. Analysis Models for the Pharmacokinetic Parameters of Metoprolol

Model	Formula	No. of θ s	LLD
1	$CL/F_i = \theta_1 \cdot WT_i \cdot (1 + \eta_{CL/F_i})$ $V/F_i = \theta_2 \cdot WT_i \cdot (1 + \eta_{V/F_i})$	2	—
2	$CL/F_i = \theta_1 \cdot WT_i^{\theta_3} \cdot (1 + \eta_{CL/F_i})$ $V/F_i = \theta_2 \cdot WT_i \cdot (1 + \eta_{V/F_i})$	3	1.25 vs. Model 1
3	$CL/F_i = \theta_1 \cdot WT_i \cdot (1 + \eta_{CL/F_i})$ $V/F_i = \theta_2 \cdot WT_i^{\theta_3} \cdot (1 + \eta_{V/F_i})$	3	0.33 vs. Model 1
4	$CL/F_i = (\theta_1 + \theta_5 \cdot G1 - \theta_5 \cdot G3) \cdot WT_i \cdot (1 + \eta_{CL/F_i})$ $V/F_i = \theta_2 \cdot WT_i \cdot (1 + \eta_{V/F_i})$	3	54.35 vs. Model 1
5	$CL/F_i = (\theta_1 + \theta_5 \cdot G1 - \theta_6 \cdot G3) \cdot WT_i \cdot (1 + \eta_{CL/F_i})$ $V/F_i = \theta_2 \cdot WT_i \cdot (1 + \eta_{V/F_i})$	4	2.65 vs. Model 4
6	$CL/F_i = (\theta_1 + \theta_5 \cdot G1 - \theta_5 \cdot G3) \cdot WT_i \cdot (1 + \eta_{CL/F_i})$ $V/F_i = (\theta_2 + \theta_7 \cdot G1 - \theta_7 \cdot G3) \cdot WT_i \cdot (1 + \eta_{V/F_i})$	4	6.36 vs. Model 4
7	$CL/F_i = (\theta_1 + \theta_5 \cdot G1 - \theta_5 \cdot G3) \cdot WT_i \cdot (1 + \eta_{CL/F_i})$ $V/F_i = (\theta_2 + \theta_7 \cdot G1 - \theta_8 \cdot G3) \cdot WT_i \cdot (1 + \eta_{V/F_i})$	5	1.27 vs. Model 6
8	$CL/F_i = (\theta_1 + \theta_5 \cdot G1 - \theta_5 \cdot G3) \cdot (1 - \theta_9 \cdot (AGE - 70)) \cdot WT_i \cdot (1 + \eta_{CL/F_i})$ $V/F_i = (\theta_2 + \theta_7 \cdot G1 - \theta_7 \cdot G3) \cdot WT_i \cdot (1 + \eta_{V/F_i})$	5	3.50 vs. Model 6
9	$CL/F_i = (\theta_1 + \theta_5 \cdot G1 - \theta_5 \cdot G3) \cdot \theta_{10}^{AGE > 70} \cdot WT_i \cdot (1 + \eta_{CL/F_i})$ $V/F_i = (\theta_2 + \theta_7 \cdot G1 - \theta_7 \cdot G3) \cdot \theta_{10}^{AGE > 70} \cdot WT_i \cdot (1 + \eta_{V/F_i})$	5	6.76 vs. Model 6
10	$CL/F_i = (\theta_1 + \theta_5 \cdot G1 - \theta_5 \cdot G3) \cdot \theta_{10}^{AGE > 70} \cdot WT_i \cdot (1 + \eta_{CL/F_i})$ $V/F_i = (\theta_2 + \theta_7 \cdot G1 - \theta_7 \cdot G3) \cdot \theta_{11}^{AGE > 70} \cdot WT_i \cdot (1 + \eta_{V/F_i})$	6	1.13 vs. Model 9
11	$CL/F_i = (\theta_1 + \theta_5 \cdot G1 - \theta_5 \cdot G3) \cdot \theta_{10}^{AGE > 70} \cdot \theta_{12}^{SEX} \cdot WT_i \cdot (1 + \eta_{CL/F_i})$ $V/F_i = (\theta_2 + \theta_7 \cdot G1 - \theta_7 \cdot G3) \cdot \theta_{10}^{AGE > 70} \cdot \theta_{12}^{SEX} \cdot WT_i \cdot (1 + \eta_{V/F_i})$	6	2.75 vs. Model 9
12	$CL/F_i = (\theta_1 + \theta_5 \cdot G1 - \theta_5 \cdot G3) \cdot \theta_{10}^{AGE > 70} \cdot WT_i \cdot (1 + \eta_{CL/F_i})$ $V/F_i = (\theta_2 + \theta_7 \cdot G1 - \theta_7 \cdot G3) \cdot \theta_{13}^{SEX} \cdot WT_i \cdot (1 + \eta_{V/F_i})$	6	0.21 vs. Model 9
13	$CL/F_i = (\theta_1 + \theta_5 \cdot G1 - \theta_5 \cdot G3) \cdot \theta_{10}^{AGE > 70} \cdot \theta_{14}^{CHF} \cdot WT_i \cdot (1 + \eta_{CL/F_i})$ $V/F_i = (\theta_2 + \theta_7 \cdot G1 - \theta_7 \cdot G3) \cdot \theta_{10}^{AGE > 70} \cdot \theta_{14}^{CHF} \cdot WT_i \cdot (1 + \eta_{V/F_i})$	6	1.19 vs. Model 9
14	$CL/F_i = (\theta_1 + \theta_5 \cdot G1 - \theta_5 \cdot G3) \cdot \theta_{10}^{AGE > 70} \cdot WT_i \cdot (1 + \eta_{CL/F_i})$ $V/F_i = (\theta_2 + \theta_7 \cdot G1 - \theta_7 \cdot G3) \cdot \theta_{15}^{CHF} \cdot WT_i \cdot (1 + \eta_{V/F_i})$	6	0.05 vs. Model 9
15	$CL/F_i = (\theta_1 + \theta_5 \cdot G1 - \theta_5 \cdot G3) \cdot \theta_{10}^{AGE > 70} \cdot \theta_{16}^{2D6*2} \cdot WT_i \cdot (1 + \eta_{CL/F_i})$ $V/F_i = (\theta_2 + \theta_7 \cdot G1 - \theta_7 \cdot G3) \cdot \theta_{10}^{AGE > 70} \cdot \theta_{16}^{2D6*2} \cdot WT_i \cdot (1 + \eta_{V/F_i})$	6	2.17 vs. Model 9
16	$CL/F_i = (\theta_1 + \theta_5 \cdot G1 - \theta_5 \cdot G3) \cdot \theta_{10}^{AGE > 70} \cdot \theta_{17}^{2C19PM} \cdot WT_i \cdot (1 + \eta_{CL/F_i})$ $V/F_i = (\theta_2 + \theta_7 \cdot G1 - \theta_7 \cdot G3) \cdot \theta_{10}^{AGE > 70} \cdot \theta_{17}^{2C19PM} \cdot WT_i \cdot (1 + \eta_{V/F_i})$	6	0.05 vs. Model 9

Table 2. Population Pharmacokinetic Parameters of Metoprolol Estimated by Model 9

Parameters	Estimates	95% CI
θ_1 (l/h/kg)	0.938	(0.835—1.04)
θ_2 (l/kg)	4.52	(3.86—5.18)
θ_5 (l/h/kg)	0.439	(0.328—0.550)
θ_7 (l/kg)	0.637	(0.153—1.12)
θ_{10}	0.739	(0.600—0.878)
$\omega_{CL/F}$ (%)	29.7	(21.7—36.1)
$\omega_{V/F}$ (%)	17.1	(0.00—25.7)
σ (ng/ml)	4.54	(1.79—6.16)

estimated to be 73.9% (θ_{10}) of that in the younger (≤ 70 years old) patients. The V/F value in Group 1 ($\theta_2 + \theta_7$) was 1.33 times as large as that in Group 3 ($\theta_2 - \theta_7$). The final $\omega_{CL/F}$ and $\omega_{V/F}$ values were 29.7% and 17.1%, respectively, which were much smaller than those for Model 1.

Figure 3 shows the effect of *CYP2D6*10*, age, gender, and cardiac function on the individual CL/F values of metoprolol for the 34 patients, where the pharmacokinetic parameters in individual patients were obtained from population estimates for Model 9 according to Bayes' theorem using the NONMEM posthoc option. Significant effects of the *CYP2D6*10* genotype and aging were observed in the individual CL/F values, whereas no significant effect of gender and cardiac function was observed (Fig. 3). Figure 4 shows the effect of *CYP2D6*10*, age, gender, and cardiac function on the individual V/F values of metoprolol in 34 patients. A significant

Fig. 3. Effect of *CYP2D6*10*, Age, Gender, and Heart Failure on the Oral Clearance of Metoprolol

Circles indicate the younger (56—70 years old) patients, and diamonds indicate the older (71—83 years old) patients in each Group. Open symbols: male patients; solid symbols: female patients. The arrows indicate the patients with NYHA class II or III. Bars indicate the mean $\pm$ S.D. for each group.

effect of the *CYP2D6*10* genotype was observed on the individual V/F values, whereas no significant effect of gender, age, and cardiac function was observed (Fig. 4). Figure 5 shows the effect of *CYP2D6*2* and the *CYP2C19* genotype on the individual CL/F values of metoprolol in 34 patients. The CL/F values in patients with *CYP2D6*2* were similar to those in patients without *CYP2D6*2*. In addition, the CL/F values for poor metabolizers of *CYP2C19* were similar to those for extensive metabolizers of *CYP2C19*.

Fig. 4. Effect of *CYP2D6*10*, Age, Gender, and Heart Failure on the Apparent Distribution Volume of Metoprolol

Circles indicate the younger (56–70 years old) patients, and diamonds indicate the older (71–83 years old) patients in each Group. Open symbols: male patients; solid symbols: female patients. The arrows indicate patients with NYHA class II or III. Bars indicate the mean $\pm$ S.D. for each group.

DISCUSSION

In this study, we measured the concentrations of metoprolol in whole blood obtained from 34 middle-aged and elderly Japanese patients, and estimated the CL/F and V/F of the drug using the NONMEM program. The present study confirmed the previous findings¹¹⁾ that the CL/F and V/F values in patients with the *CYP2D6*10* allele were significantly lower than those in patients without it (Table 1, Figs. 2, 3). In addition, the CL/F value in the older (>70 years old) patients was lower than in the younger (≤ 70 years old) patients (Table 1, Fig. 2). On the other hand, the *CYP2D6*2* allele, *CYP2C19* genotype, gender, and heart failure showed no significant effects on the pharmacokinetics of metoprolol (Table 1, Figs. 2–4). The mean pharmacokinetic parameters of metoprolol estimated in the present study were comparable with those in the previous report,¹¹⁾ although the considerable interindividual variability was observed in the B/P ratio of the drug (Table 2, Fig. 1).

Fractional systemic bioavailability (F) is determined by the fraction of drugs absorbed from the gastrointestinal tract (F_{abs}) and the fraction that escapes hepatic first-pass extraction (F_h) as follows:

$$F = F_{\text{abs}} \cdot F_h \quad (4)$$

Metoprolol is virtually completely absorbed from the gastrointestinal tract after oral administration, and is metabolized almost exclusively in the liver.^{12,13)} Therefore, assuming that the gastrointestinal absorption is complete ($F_{\text{abs}} = 1$), F can be described by the following equation according to the well-stirred model²⁴⁾:

$$F = F_h = \frac{Q_h}{Q_h + f_u \cdot CL_{\text{int}}} \quad (5)$$

where Q_h is hepatic blood flow, f_u is the unbound fraction of metoprolol in the blood, and CL_{int} is the intrinsic hepatic clearance of the drug. In addition, assuming that there is no extrahepatic metabolism (excretion), CL/F can be described by the following equation²⁴⁾:

$$CL/F = f_u \cdot CL_{\text{int}} \quad (6)$$

Fig. 5. Effects of *CYP2D6*2* and the *CYP2C19* Genotypes on the Oral Clearance of Metoprolol

Circles indicate the younger (56–70 years old) patients, and diamonds indicate the older (71–83 years old) patients in each Group. Open symbols: *CYP2D6*1/*1* (Group 1a), *CYP2D6*1/*10* (Group 2a), or *CYP2D6*10/*10* (Group 3); solid symbols: *CYP2D6*1/*2* (Group 1b) or *CYP2D6*2/*10* (Group 2b). The arrows indicate poor metabolizers of *CYP2C19*. Bars indicate the mean $\pm$ S.D. for each group.

Since the bound fraction of metoprolol in human plasma is only 12%,¹³⁾ the alteration of plasma protein binding of the drug would have little influence on the CL/F value. In contrast, a reduced CL_{int} value caused by *CYP2D6*10* and aging can lead to a decrease in the CL/F value. Fukuda *et al.* suggested that decreased drug clearance due to *CYP2D6*10* could be caused by the low expression and affinity of *CYP2D6*.²⁵⁾ In addition, Castleden *et al.* suggested that hepatic metabolism and extraction during the first pass of the *CYP2D6* substrate, propranolol, is decreased by aging.^{26,27)}

Ward *et al.* reported that 4-hydroxylation of propranolol, structurally similar to metoprolol, is catalyzed by *CYP2D6*, but side-chain oxidation of the drug is catalyzed in part by *CYP2C19*.²⁸⁾ Consequently, the metabolic clearance of side-chain oxidation of propranolol was 55% less in the poor metabolizers of *CYP2C19* than in the extensive metabolizers of *CYP2C19*.²⁸⁾ On the other hand, metoprolol is mainly metabolized by the three routes of *O*-demethylation, α -hydroxylation, and *N*-dealkylation, and these metabolites are excreted in the urine.²⁹⁾ *N*-Dealkylation of metoprolol is mediated by *CYP2D6* and also *CYP2C19*.¹⁷⁾ However, Regårdh and Johnsson reported that *O*-demethylation and α -hydroxylation are mediated by *CYP2D6*, and that the metabolites formed by these mechanisms account for 70% of the urinary recovery of metoprolol doses.¹³⁾ These reports suggest that metoprolol is mainly metabolized by *CYP2D6*, which is consistent with the present finding that *CYP2C19* genotype showed no significant effect on the CL/F values of metoprolol (Table 1, Fig. 4).

Table 3 summarizes the CL/F and V/F values in a typical patient for Groups 1, 2, and 3, where the pharmacokinetic parameters in the younger (≤ 70 years old) and also older (>70 years old) patients were obtained from population estimates for Model 9 (Table 2). The CL/F and V/F values listed in Table 3 were almost identical to the mean CL/F and V/F values shown in Figs. 3 and 4. In the present study, the hepatic clearance of metoprolol could be interpreted physiologically, because the blood metoprolol concentrations were used for the pharmacokinetic analysis.^{14,24)} Then, the F values were estimated using Eqs. 5 and 6, assuming that the Q_h value in a typical younger (≤ 70 years old) and older (>70

Table 3. Physiological Pharmacokinetic Parameters of Metoprolol

Parameters		Group 1		Group 2		Group 3	
		56≤AGE≤70	70<AGE≤83	56≤AGE≤70	70<AGE≤83	56≤AGE≤70	70<AGE≤83
CL/F	(l/h/kg)	1.38	1.02	0.938	0.693	0.499	0.369
V/F	(l/kg)	5.16	5.16	4.52	4.52	3.88	3.88
Q _h	(l/h/kg)	1.29	1.02	1.29	1.02	1.29	1.02
F		0.484	0.500	0.579	0.595	0.721	0.734
CL	(l/h/kg)	0.666	0.509	0.543	0.413	0.360	0.271
V	(l/kg)	2.49	2.58	2.62	2.69	2.80	2.85

years old) patient is 1.29 l/h/kg and 1.02 l/h/kg, respectively.³⁰⁾ In addition, the systemic clearance (CL) and volume of distribution (V) were calculated as follows:

$$CL = F \cdot CL/F \quad (7)$$

$$V = F \cdot V/F \quad (8)$$

Table 3 also lists the Q_h , F , CL , and V values for Groups 1, 2, and 3. The calculated F values for Group 1 were significantly lower than those for Group 2, and the calculated F values for Group 3 were significantly higher than those for Group 2. Interestingly, no significant difference in the F values was observed between the younger (≤70 years old) and older (>70 years old) patients. In contrast, the calculated CL values for Group 1 were higher than those for Group 2, and the calculated CL values for Group 3 were lower than those for Group 2. The calculated CL values in the younger (≤70 years old) patients were higher than those in older (>70 years old) patients (Table 3). Accordingly, we thought that the significant effect of the *CYP2D6**10 genotype on the CL/F value was probably due not only to the decrease in CL but also to the increase in F . On the other hand, the significant effect of aging on CL/F was thought to be mainly due to the decrease in CL . In addition, we thought that the increase in F was mainly responsible for the significant effect of the *CYP2D6**10 genotype on the V/F value, because it was unlikely that a genetic mutation in *CYP2D6* would affect V of the drug.^{11,31)} However, the calculated V slightly fluctuated by the *CYP2D6**10 genotype, which might be attributed to estimation errors in the CL/F and/or V/F values (Table 3). That is, approximation errors could lie in the assumptions that the disposition of orally administered metoprolol is described by the one-compartment model with bolus input, that the gastrointestinal absorption of the drug is complete, that non-hepatic elimination of the drug is negligible, or that the hepatic extraction process is described by the well-stirred model.^{12,14,19)}

Metoprolol has been administered to Caucasian patients with heart failure at the dose range of 12.5–200 mg/d,^{32,33)} and with hypertension at the dose range of 75–450 mg/d.^{34,35)} In the present study, middle-aged and elderly Japanese patients were routinely treated at a lower dose range of 40–120 mg/d, and no adverse effect of metoprolol was observed in Groups 1, 2, and 3. However, careful dose titration is necessary to treat Japanese patients at the relatively higher doses of metoprolol, because the gene frequency of *CYP2D6**10 in Japanese is higher than that in Caucasians.^{1,8–10)} In addition, the genotyping of *CYP2D6* may be useful in designing the dosage of metoprolol for Japanese patients and in determining which patients are at risk for ad-

verse effects.

In conclusion, we systematically estimated the variability of the pharmacokinetics of metoprolol in routinely treated middle-aged and elderly Japanese patients using the NON-MEM approach. Our findings indicate that the *CYP2D6**10 allele and aging are the major factors that affects the pharmacokinetic variability of metoprolol. The results also suggest that the pharmacokinetics of metoprolol in Japanese extensive metabolizers of *CYP2D6* is different from that in Caucasian extensive metabolizers because of the large interethnic difference in the gene frequency of *CYP2D6**10.

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REFERENCES

- Bradford L. D., *Pharmacogenomics*, **3**, 229–243 (2002).
- Bertilsson L., Lou Y. Q., Du Y. L., Liu Y., Kuang T. Y., Liao X. M., Wang K. Y., Reviriego J., Selius L., Sjöqvist F., *Clin. Pharmacol. Ther.*, **51**, 388–397 (1992).
- Nakamura K., Goto F., Ray W. A., McAllister C. B., Jacqz E., Wilkinson G. R., Branch R. A., *Clin. Pharmacol. Ther.*, **38**, 402–408 (1985).
- Huang J. D., Chuang S. K., Cheng C. L., Lai M. L., *Clin. Pharmacol. Ther.*, **65**, 402–407 (1999).
- Johansson I., Lundqvist E., Bertilsson L., Dahl M. L., Sjöqvist F., Ingerman S. M., *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 11825–11829 (1993).
- Yokota H., Tamura S., Furuya H., Kimura S., Watanabe M., Kanazawa I., Kondo I., Gonzalez F. J., *Pharmacogenetics*, **3**, 256–263 (1993).
- Johansson I., Oscarson M., Yue Q. Y., Bertilsson L., Sjöqvist F., Ingman S. M., *Mol. Pharmacol.*, **46**, 452–459 (1994).
- Tateishi T., Chida M., Ariyoshi N., Mizorogi Y., Kamataki T., Kobayashi S., *Clin. Pharmacol. Ther.*, **65**, 570–575 (1999).
- Nishida Y., Fukuda T., Yamamoto I., Azuma J., *Pharmacogenetics*, **10**, 567–570 (2000).
- Roh H. K., Chung J. Y., Oh D. Y., Park C. S., Svensson J. O., Dahl M. L., Bertilsson L., *Br. J. Clin. Pharmacol.*, **52**, 265–271 (2001).
- Taguchi M., Nozawa T., Kameyama T., Inoue H., Takesono C., Mizukami A., Hashimoto Y., *Eur. J. Clin. Pharmacol.*, **59**, 385–388 (2003).
- Regårdh C. G., Borg K. O., Johansson R., Johansson G., Palmer L., *J. Pharmacokinet. Biopharm.*, **2**, 347–364 (1974).
- Regårdh C. G., Johnsson G., *Clin. Pharmacokinet.*, **5**, 557–569 (1980).
- Wilkinson G. R., Shand D. G., *Clin. Pharmacol. Ther.*, **18**, 377–390 (1975).
- Regårdh C. G., Landahl S., Larsson M., Lundborg P., Steen B., Hoffmann K. J., Lagerström P. O., *Eur. J. Clin. Pharmacol.*, **24**, 221–226 (1983).
- Kendall M. J., Brown D., Yates R. A., *Br. J. Clin. Pharmacol.*, **4**, 497–499 (1977).
- Rendic S., *Drug. Metab. Rev.*, **34**, 83–448 (2002).

- 18) Luzier A. B., Killian A., Wilton J. H., Wilson M. F., Forrest A., Kazierad D. J., *Clin. Pharmacol. Ther.*, **66**, 594—601 (1999).
- 19) Johnsson G., Regårdh C. G., Sölvell L., *Acta Pharmacol. Toxicol.*, **36** (Suppl. V), 31—44 (1975).
- 20) Lamba J. K., Dhiman R. K., Kohli K. K., *Clin. Pharmacol. Ther.*, **68**, 328—335 (2000).
- 21) Hamelin B. A., Bouayad A., Méthot J., Jobin J., Desgagnés P., Poirier P., Allaire J., Dumesnil J., Turgeon J., *Clin. Pharmacol. Ther.*, **67**, 466—477 (2000).
- 22) Sheiner L. B., Beal S. L., *J. Pharmacokin. Biopharm.*, **11**, 303—319 (1983).
- 23) Beal S. L., Boeckmann A. J., Sheiner L. B., “NONMEM Users Guides: NONMEM Project Group,” University of California, San Francisco, 1992.
- 24) Wilkinson G. R., *Pharmacol. Rev.*, **39**, 1—47 (1987).
- 25) Fukuda T., Nishida Y., Imaoka S., Hiroi T., Naohara M., Funae Y., Azuma J., *Arch. Biochem. Biophys.*, **380**, 303—308 (2000).
- 26) Castleden C. M., Kaye C. M., Parsons R. L., *Br. J. Clin. Pharmacol.*, **2**, 303—306 (1975).
- 27) Castleden C. M., George C. F., *Br. J. Clin. Pharmacol.*, **7**, 49—54 (1979).
- 28) Ward S. A., Walle T., Walle U. K., Wilkinson G. R., Branch R. A., *Clin. Pharmacol. Ther.*, **45**, 72—79 (1989).
- 29) Borg K. O., Carlsson E., Hoffmann K. J., Jönsson T. E., Thorin H., Wallin B., *Acta Pharmacol. Toxicol.*, **36**, 125—135 (1975).
- 30) Zoli M., Magalotti D., Bianchi G., Gueli C., Orlandini C., Grimaldi M., Marchesini G., *Age and Ageing*, **28**, 29—33 (1999).
- 31) Kirchheiner J., Müller G., Meineke I., Wernecke K. D., Roots I., Brockmöller J., *J. Clin. Psychopharmacol.*, **23**, 459—466 (2003).
- 32) MERIT-HF study group, *Lancet*, **353**, 2001—2007 (1999).
- 33) Poole-Wilson P. A., Swedberg K., Cleland J. G., Di Lenarda A., Hanrath P., Komajda M., Lubsen J., Lutiger B., Metra M., Remme W. J., Torp-Pedersen C., Scherhag A., Skene A., Carvedilol Or Metoprolol European Trial Investigators, *Lancet*, **362**, 7—13 (2003).
- 34) Bergstrand R. H., Vedin J. A., Wilhelmsson C. E., Berglung G., *Eur. J. Clin. Pharmacol.*, **10**, 375—379 (1976).
- 35) Tuomilehto J., Pohjola M., *Ann. Clin. Res.*, **10**, 24—29 (1978).