Table II Final Population Pharmacokinetic Model Formulated Based on Data Obtained From 91 Patients After Intravenous Administration of Pilsicainide

Parameter Symbol	Annotation	Value	SE
Fixed effects[a]			
θ_1, L/min/kg	CCR coefficient in Fac	3.28	0.582
θ_2	Female coefficient in clearance	0.498	0.0681
V_c, L/kg	Volume of central compartment	0.0775	0.0190
Q, L/min	Intercompartmental clearance	1.73	0.318
θ_3, L/kg	Intercept of volume of peripheral compartment	0.783	0.119
θ_4	AGE coefficient in volume of peripheral compartment	0.00627	0.00190
Random effects[b]			
ω^2_{CL}	Interindividual variance of CL_j	0.0198	0.0182
ω^2_{Vc}	Interindividual variance of V_{cj}	0.101	0.118
ω^2_{Q}	Interindividual variance of Q_j	0.175	0.0811
ω^2_{Vp}	Interindividual variance of V_{pj}	0.0635	0.0168
σ^2	Intraindividual variance of C_{ij}	0.0339	0.00888

SE = standard error of mean for estimation; CCR = predicted creatinine clearance (L/min/kg); AGE = age (years); CL_{TV} = typical value of clearance (CL) in the population (L/min/kg); SEX = 0 for male and 1 for female; V_{pTV} = typical value of volume of peripheral compartment (V_p) in the population (L/kg); P_j = estimated pharmacokinetic parameter in the jth individual; P_{TV} = typical value of a pharmacokinetic parameter in the population; ηP_j = jth interindividual variability in P_{TV}; C_{ij} and $C_{pred,ij}$ = ith observed and estimated plasma concentrations of pilsicainide in the jth individual, respectively; ε_{ij} = ith residual variability in plasma concentration of pilsicainide in the jth individual.

a. $Fac = \theta_1 \cdot CCR$; $CL_{TV} = Fac \cdot (1 - SEX) + \theta_2 \cdot Fac \cdot SEX$; $V_{pTV} = \theta_3 + \theta_4 \cdot AGE$.

b. $P_j = P_{TV} \cdot (1 + \eta P_j)$; $C_{ij} = C_{pred,ij} \cdot (1 + \varepsilon_{ij})$.

CL, V_p) estimated by our population PK analysis (Figure 1, Table II) agree well with those reported in the conventional PK studies.[3,8,13] The reason female patients possess significantly ($P < .01$) lower body-weight-normalized CL than male patients do (Table II) remains unclear. However, we are tempted to speculate that a gender difference in the activity of certain unidentified renal OCTs may be associated with our finding. Involvement of active renal tubular secretion of the drug has been strongly suggested by previous studies[3,8,13] because the renal CL of pilsicainide is approximately 2 times greater than creatinine clearance in healthy subjects. Shiga et al[8] demonstrated that the renal elimination of pilsicainide is interfered with significantly by the coadministration of cimetidine, which has been shown to inhibit active tubular excretion of various cationic drugs (eg, procainamide[14] and metformin[15]). Since pilsicainide is a cationic compound, certain OCTs may be involved in its renal elimination. To our knowledge, no data are available on whether gender difference exists in the OCT activities in human renal tubular cells. However, previous studies[16-19] demonstrated that female rats have a substantially lower OCT activity for tetraethyl ammonium and other cationic small molecules and lower level of mRNA expression of OCT2, but not of OCT1, than male rats in renal tubular cells. Similarly, the activity of the renal organic anion transporter (OAT) in female rats is also significantly less than in male rats.[20,21] Recently, female patients have been shown to have a 50% less systemic CL of telmisartan, an angiotensin II receptor antagonist, than do male patients.[22] The drug and its conjugate are claimed to be excreted primarily into bile via an OAT, canalicular multispecific OAT.[23]

The sequential population PK/PD analysis using the effect compartment model revealed that the patients who exhibited a BrS-like ECG pattern (coved or saddleback ST-segment elevation >1.5 mV) after pilsicainide administration (responders) also showed significantly ($P < .01$) greater prolongation of PQ and PEQ intervals compared to those who did not exhibit the ECG pattern (nonresponders). E_{max} values of ΔPQ and ΔPEQ in the responders were 40% and 50%, respectively, greater than those in the nonresponders (Table III, Figure 3). These findings are consistent with previous studies[5,7,24,25] conducted in patients diagnosed with BrS based on ECG responses elicited by the administration of various class I antiarrhythmics (flecainide, disopyramide, and mexiletine), using similar diagnostic criteria as employed in the present study. Our data suggest that pilsicainide-induced ST-segment elevations mimicking BrS and greater prolongation of intracardiac conduction may be attributable to exaggerated responsiveness of sodium channels to pilsicainide. Since BrS is a sodium channelopathy, these parameters may serve as useful tools for probing

Table III Final Population Pharmacodynamic Model for the Relationship Between Effect Site Pilsicainide Concentrations and Electrocardiogram Parameters Associated With Intracardiac Conduction (n = 77)

Pharmacodynamic Parameter	ΔP	ΔPQ	ΔPEQ	ΔQRS
Number of pharmacodynamic data points	296	296	296	352
Error model				
Interindividual	Proportional	Additive	Proportional	Proportional
Residual	Additive	Proportional	Additive	Proportional
ΔOBJ	18.428	54.971	12.101	81.604
Fixed effects				
K_{e0TV}, min^{-1}	0.330 (0.0981)	0.0756 (0.0118)	0.130 (0.0609)	0.0108 (0.0321)
$K_{e0TV} + \theta$/AGE	—	—	—	1.33 (2.08)
$K_{e0TV} \bullet (1 - STE) + \theta \bullet K_{e0TV} \bullet STE$	—	—	—	9.67 (4.20)
E_{maxTV}, ms	46.6 (16.1)	73.2 (11.2)	26.2 (9.37)	26.4 (24.7)
$E_{maxTV} + \theta \bullet AGE$	—	—	—	3.58 (0.324)
$E_{maxTV} \bullet (1 - SEX) + \theta \bullet E_{maxTV} \bullet SEX$	—	1.34 (0.156)	—	1.55 (0.282)
$E_{maxTV} \bullet (1 - STE) + \theta \bullet E_{maxTV} \bullet STE$	—	1.38 (0.135)	1.48 (0.237)	—
EC_{50TV}, μg/mL	13.7 (13.0)	4.19 (1.31)	0.675 (0.354)	4.97 (11.0)
$EC_{50TV} + \theta \bullet AGE$	−0.174 (0.167)	−0.0504 (0.0181)	—	—
γ_{TV}	0.577 (0.111)	0.828 (0.0663)	1.61 (1.25)	0.658 (0.173)
Random effects				
ω^2_{Ke0}	9.59 (8.70)	2.18E-9 (0.00183)	1.12 (0.988)	25.1 (12.0)
ω^2_{Emax}	0.347 (0.160)	743 (438)	0.370 (0.100)	0.361 (0.566)
ω^2_{EC50}	1.66E-8 (2.50)	0.468 (0.574)	1.34E-8 (0.132)	9.06E-5 (3.54)
ω^2_{γ}	0.294 (0.218)	0.215 (0.127)	0.382 (0.978)	0.343 (0.336)
σ^2	40.4 (10.6)	0.0503 (0.0136)	53.0 (10.5)	0.140 (0.0378)

Data are presented as population mean (SE). ΔOBJ = decrement of objective function value from basic model; K_{e0TV}, E_{maxTV}, EC_{50TV}, and γ_{TV} = the typical values of effect site elimination rate constant (min^{-1}), maximum effect (ms), effect site pilsicainide concentrations associated with a 50% E_{max} (μg/mL), and shape parameter (ie, Hill coefficient) in the patient population; ω^2_x = the interindividual variance of each parameter; σ^2 = the residual variance of pharmacodynamic response; AGE = age (years); STE = 0 for nonresponder and 1 for responder about >0.15 mV ST-segment elevation after intravenous pilsicainide administration; SEX = 0 for male and 1 for female; SE = standard error of mean for estimation.

sodium channel function in patients who have arrhythmias suspected of BrS.

Our data should be interpreted with caution because of the limitations described below. First, our patients were not homogenous regarding demographic and clinical backgrounds. For instance, patients with atrial fibrillation (group C) were significantly older and had lower CL_{cr} compared to those suspected of BrS (groups A and B, Table I). In addition, female patients were more prevalent (36%) in the atrial fibrillation group than in groups A and B (16%). Since we analyzed the population PK of pilsicainide by incorporating age, CL_{cr}, and gender as independent covariates, we consider that our conclusion would be tenable for the bias associated with patient heterogeneity described above. Nevertheless, we cannot categorically deny the possibility that atrial fibrillation has a unique effect on the PK of pilsicainide independent of age, CL_{cr}, and gender. In this context, further studies are required to clarify this issue. Second, because the number of patients who participated in our population PK and PD study was relatively small, it was not feasible to undertake model validation to assess stability and performance using a data-splitting method. Thus, further studies are necessary to confirm our findings using a larger number of subjects. Finally, there are fundamental difficulties in the accurate diagnosis of BrS. All patients who participated in the present study had a family history of cardiac sudden death, syncope episodes, and right bundle branch block coupled with typical or marginal ST-segment elevations in ECG at rest despite structurally normal hearts. ST-segment elevation of >+0.15 or +0.20 mV after the administration of class I antiarrhythmic agents has been proposed as a diagnostic criterion of BrS,[7] while its sensitivity and specificity remain to be confirmed by a large clinical trial. While many genetic mutations of *SCN5A* have been implicated in the arrhythmogenicity of BrS,[26-28] such mutations are detected in at most 20% of the patients who met the above diagnostic criteria of BrS.[29,30] Therefore, caution must

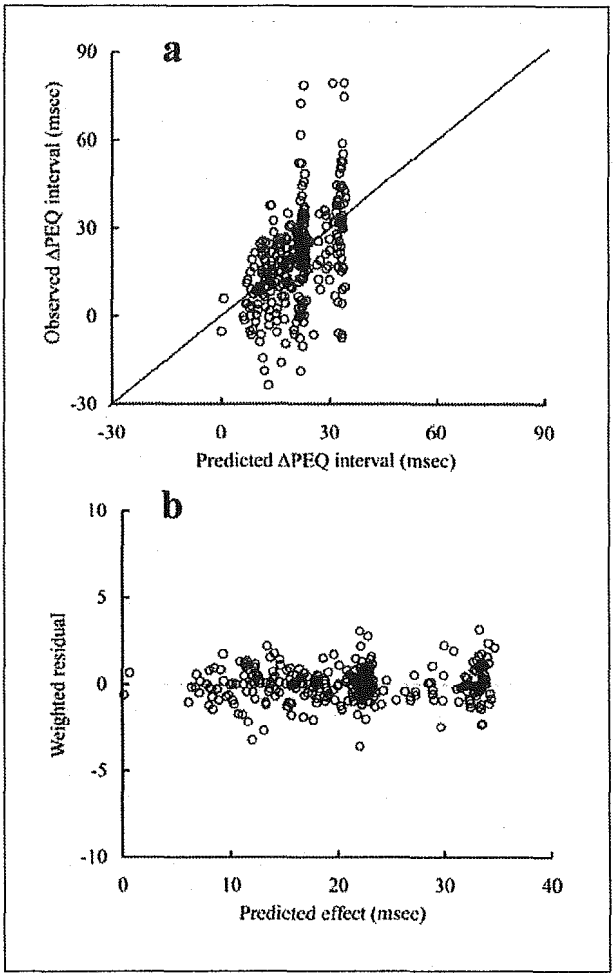

Figure 2. (a) Scatter plots of the observed ΔPEQ interval versus the interval predicted by the final population PK/PD model, with reference to the line of unity (y = x). (b) Scatter plots of weighted residuals of the ΔPEQ interval versus the final population PK/PD model-predicted values.

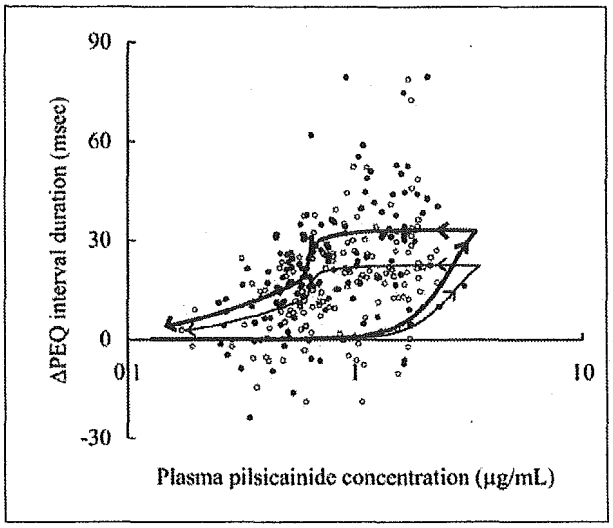

Figure 3. Relationships between plasma pilsicainide concentrations and prolongation of PEQ interval relative to the baseline values (ie, ΔPEQ) in patients after intravenous infusion of pilsicainide. Thick and thin curves represent the typical plasma drug concentration-response relationship with time-sequence depicted by arrows in the patients who developed the exaggerated ST-segment elevation (●) and those who did not (○). Note that the phenotypic trait of the ST-segment elevation >0.15 mV from the baseline tracing is a significant (P < .05) covariate for the maximum prolongation of ΔPEQ (E_{max}). Detailed descriptions of the population PK/PD analysis are given in the text.

be exercised in interpreting our data in the light of diagnostic usefulness, particularly in association with genetic mutations of *SCN5A*, until a more satisfactory method for molecular diagnosis of BrS becomes available.

In conclusion, we have performed a population PK analysis of the pure sodium channel blocker pilsicainide in patients with cardiac arrhythmias. We found that gender and CL_{cr} are independent covariates associated with systemic clearance of the drug. The population PD analysis revealed that the patients who developed BrS-like ST-segment elevation after the administration of pilsicainide also have a greater dromotropic effect in PQ and PEQ intervals than those who did not. Our data further support the idea that ST-segment elevations observed in patients with BrS may be associated with greater susceptibility of sodium channels to class IC antiarrhythmics.

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REFERENCES

1. Hattori Y, Inomata N, Aisaka K, Ishihara T. Electrophysiological actions of N-(2, 6-dimethylphenyl)-8-pyrrolizidine-acetamide hydrochloride hemihydrate (SUN1165), a new antiarrhythmic agent. *J Cardiovasc Pharmacol.* 1986;8:998-1002.

2. Hattori Y, Inomata N. Modes of the Na channel blocking action of pilsicainide, a new antiarrhythmic agent, in cardiac cells. *Jpn J Pharmacol.* 1992;58:365-373.

3. Nakashima M, Kanamaru M. Phase I study of pilsicainide hydrochloride (SUN1165) injection. *J Clin Ther Med.* 1998;14:47-61.

4. Antzelevitch C, Brugada P, Borggrefe M, et al. Brugada syndrome: report of the second consensus conference. *Circulation.* 2005;111:659-670.

5. Miyazaki T, Mitamura H, Miyoshi S, Soejima K, Ogawa S, Aizawa Y. Autonomic and antiarrhythmic drug modulation of ST segment elevation in patients with Brugada syndrome. *J Am Coll Cardiol.* 1996;27:1061-1070.

6. Scheiner LB, Rosenberg B, Marathe VV. Estimation of population characteristics of pharmacokinetic parameters from routine clinical data. *J Pharmacokinet Biopharm.* 1977;5:445-479.

7. Brugada R, Brugada J, Antzelevitch C, et al. Sodium channel blockers identify risk for sudden death patients with ST-segment elevation and right bundle branch block but structurally normal hearts. *Circulation.* 2000;101:510-515.

8. Shiga T, Hashiguchi M, Urae A, Kasanuki H, Rikihisa T. Effect of cimetidine and probenecid on pilsicainide renal clearance in humans. *Clin Pharmacol Ther.* 2000;67:222-228.

9. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron.* 1976;16:31-41.

10. Parke J, Holford NH, Charles BG. A procedure for generating bootstrap samples for the validation of nonlinear mixed-effects population models. *Comput Methods Programs Biomed.* 1999:59;19-29.

11. Sheiner LB, Stanski DR, Vozeh S, Miller RD, Ham J. Simultaneous modeling of pharmacokinetics and pharmacodynamics: application to d-tubocurarine. *Clin Pharmacol Ther.* 1979;25:358-371.

12. Ailings M, Wilde A. Brugada syndrome: clinical data and suggested pathophysiological mechanism. *Circulation.* 1999;99:666-673.

13. Konishi T, Bito K, Matsumura T, Kanamaru M. The clinical pharmacokinetics and antiarrhythmic effect of intravenous pilsicainide hydrochloride [SUN1165 (inj)]. *J Clin Ther Med.* 1998;14:107-121.

14. Somogyi A, McLean A, Heinzow B. Cimetidine-procainamide pharmacokinetic interaction in man: evidence of competition for tubular secretion of basic drugs. *Eur J Clin Pharmacol.* 1983;25:339-345.

15. Somogyi A, Stockley C, Keal J, Rolan P, Bochner F. Reduction of metformin renal tubular secretion by cimetidine in man. *Br J Clin Pharmacol.* 1987;23:545-551.

16. Kleinman LI, Lowenstein MS, Goldstein L. Sex difference in the transport of p-aminohippurate by the rat kidney. *Endocrinology.* 1966;78:403-406.

17. Bowman HM, Hook JB. Sex differences in organic ion transport by rat kidney. *Proc Soc Exp Biol Med.* 1972;141:258-262.

18. Urakami Y, Nakamura N, Takahashi K, et al. Gender differences in expression of organic cation transporter OCT2 in rat kidney. *FEBS Lett.* 1999;461:339-342.

19. Urakami Y, Okuda M, Saito H, Inui K. Hormonal regulation of organic cation transporter OCT2 expression in rat kidney. *FEBS Lett.* 2000;473:173-176.

20. Reyes JL, Melêndez E, Alegría A, Jaramillo-Juarez F. Influence of sex differences on the renal secretion of organic anions. *Endocrinology.* 1998;139:1581-1587.

21. Kato Y, Kuge K, Kusuhara H, Meier PJ, Sugiyama Y. Gender difference in the urinary excretion of organic anions in rats. *J Pharmacol Exp Ther.* 2003;302:483-489.

22. Micardis (telmisartan) [product information]. Ridgefield, Conn: Boehringer Ingelheim Pharmaceuticals; 1998.

23. Nishino A, Kato Y, Igarashi T, Sugiyama Y. Both cMOAT/MRP2 and another unknown transporter(s) are responsible for the biliary excretion of glucuronide conjugate of the nonpeptide angiotensin II antagonist, telmisartan. *Drug Metab Dispos.* 2000;28:1146-1148.

24. Shimizu W, Antzelevitch C, Suyama K, et al. Effect of sodium channel blockers on ST segment, QRS duration, and corrected QT interval in patients with Brugada syndrome. *J Cardiovasc Electrophysiol.* 2000;11:1320-1329.

25. Takenaka S, Kusano KF, Hisamatsu K, et al. Relatively benign clinical course in asymptomatic patients with Brugada-type electrocardiogram without family history of sudden death. *J Cardiovasc Electrophysiol.* 2001;21:2-6.

26. Chen Q, Kirsch GE, Zhang D, et al. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. *Nature.* 1998;392:293-296.

27. Vatta M, Dumaine R, Varghese G, et al. Genetic and biophysical basis of sudden unexplained nocturnal death syndrome (SUNDS), a disease allelic to Brugada syndrome. *Hum Mol Genet.* 2002;11:337-345.

28. Vatta M, Dumaine R, Antzelevitch C, et al. Novel mutations in domain I of *SCN5A* cause Brugada syndrome. *Mol Genet Metab.* 2002;75:317-324.

29. Towbin JA. Molecular genetic basis of sudden cardiac death. *Cardiovasc Pathol.* 2001;10:283-295.

30. Brugada R, Roberts R. Genetic aspects of arrhythmias. *Am J Med Genet.* 2000;97:310-318.

In Vivo Metabolic Activity of CYP2C19 and CYP3A in Relation to CYP2C19 Genetic Polymorphism in Chronic Liver Disease

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*To study whether chronic liver disease (CLD) and genetic polymorphism affect the hepatic activity of cytochrome P450 (CYP) isoforms, we compared in vivo CYP2C19 and CYP3A activities using 3-hour omeprazole hydroxylation index (plasma concentration ratio of omeprazole to its 5-hydroxylated metabolite; a higher index indicates lower CYP2C19 activity) and partial formation clearance of cortisol to 6β-hydroxycortisol ($CL_{cortisol \rightarrow 6\beta\text{-}HC}$) in 31 CLD patients (9 with chronic hepatitis; 22 with cirrhosis comprising 20 Child-Pugh type A, 1 type B, and 1 type C) and 30 healthy subjects with different CYP2C19 genotypes. The mean (±SEM) omeprazole hydroxylation index in CLD patients with homozygous extensive metabolizer (EM) genotype (*1/*1, n = 8), heterozyous EM (*1/*2, n = 11; *1/*3, n = 6) genotypes and poor metabolizer (PM) genotypes (*2/*2, n = 3; *3/*3, n = 3) were 17.15 ± 2.12, 20.02 ± 2.63, and 26.04 ± 3.15, respectively, which were significantly higher compared with control subjects with the corresponding CYP2C19 genotypes (0.81 ± 0.09, 1.55 ± 0.20, and 15.5 ± 1.52). CLD patients with PM genotype had significantly (P < .05) higher omeprazole hydroxylation indexes than did those with homozygous EM genotype, and those with heterozygous EM genotypes had intermediate values. The mean $CL_{cortisol \rightarrow 6\beta\text{-}HC}$ decreased significantly (P < .001) in CLD patients compared with control subjects (1.19 ± 0.12 versus 2.26 ± 0.24 mL/min). Multiple regression analysis showed that CLD, serum albumin level, and CYP2C19 genotype correlated significantly (P < .05) with the omeprazole hydroxylation index, whereas no significant correlation was observed between $CL_{cortisol \rightarrow 6\beta\text{-}HC}$ and other variables, except CLD. Because CLD and genetic polymorphism of CYP2C19 act additively to reduce CYP2C19 activity, genotyping these patients may be of value in averting adverse reactions of drugs that depend on CYP2C19 for elimination.*

Keywords: *Chronic liver disease; cirrhosis; cytochrome P450; CYP2C19; CYP3A*

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Chronic liver disease (CLD) including cirrhosis, irrespective of the cause, is associated with a reduced number of functional hepatocytes and the development of a pathological intrahepatic portosystemic shunt. As a result, the systemic clearance of drugs that undergo hepatic metabolism is often reduced in patients with CLD. Drug clearance *via* oxidative drug metabolism (mainly cytochrome P450 [CYP]) is known to be reduced in CLD.[1] Two studies have demonstrated the *in vivo* metabolic activities of 3 major CYP isoforms in patients with mild to moderate CLD,[2,3] using mephenytoin, dapsone, and debrisoquin as the model substrates for CYP2C19, CYP3A4, and CYP2D6, respectively. These studies reported a preferential reduction of CYP2C19 in CLD patients (77% from the healthy controls) compared with CYP3A4 (28%) and CYP2D6 (4%). Several other studies have also examined the differential effect of liver disease on CYP activity.[4-7] Although the existence of such effects of CLD is clinically important in the prescription of drugs in

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Table I Demographic and Clinical Characteristic of the Healthy Subjects and Patients With Chronic Liver Disease Enrolled in the Present Study

	Patients With Chronic Hepatitis	Patients With Cirrhosis	Healthy Controls
No. of subjects or patients	9	22	30
Gender, male/female	8/1	14/8	18/12
Age, y	66 ± 9.5*	70.8 ± 6.1*	50.6 ± 16.4
Child-Pugh score[a]	—	20/1/1	NA
AST, IU/L	47.5 ± 36.8*	78.5 ± 27.1*	25.7 ± 14.1
ALT, IU/L	58.4 ± 65.5*	61.4 ± 23.0*	26.9 ± 8.0
Total protein, g/dL	7.9 ± 0.6	7.4 ± 0.7	7.5 ± 0.5
Albumin, g/dL	4.3 ± 0.5	3.7 ± 0.5*	4.4 ± 0.4
Total bilirubin, mg/dL	0.5 ± 0.2	1.6 ± 3.3	0.6 ± 0.3
Prothrombin index, %	92.6 ± 9.1	77.8 ± 11.5**	ND
Creatinine, mg/dL	0.7 ± 0.22	0.9 ± 0.31	0.8 ± 0.24

Data are mean ± SD; all laboratory data shown are assayed in serum; NA = not applicable; AST = asparate aminotransferase; ALT = alanine aminotransferase; ND = not determined.
a. Classification reported by Pugh et al.[14]
*$P < .05$ compared with control.
**$P < .05$ compared with chronic hepatitis; gender difference was not significant among 3 groups by χ^2 analysis.

these patients, no subsequent studies have confirmed this finding.

Besides CLD, genetic polymorphism is another important covariate of CYP activity in certain isoforms.[8] Because CYP2C19 is involved in the hepatic metabolism of many therapeutically important drugs, its metabolic activity is under control of genetic polymorphism. At present, as many as 15 variant isoforms have been reported and some of them (eg, CYP2C19*2 and CYP2C19*3 alleles) have been shown to be associated with defective enzyme function.[9] Previous studies[10,11] have shown that the possession of either the CYP2C19*2 or the CYP2C19*3 allele is associated with reduced *in vivo* CYP2C19 activity, depending on the gene dose; the CYP2C19 activity is lowest in the poor metabolizer (PM) genotypes (CYP2C19*2/*2, *2/*3, or *3/*3), followed by the heterozygous EM genotypes (CYP2C19*1/*2 or *1/*3) and then the homozygous EM genotype (CYP2C19*1/*1). Because the prevalence of defective alleles (CYP2C19*2 and *3) in Asians, including Japanese, is greater than that in whites,[9,12,13] it is importance to study whether the genetic polymorphism of CYP2C19 would have an additive effect on the *in vivo* CYP2C19 activity, particularly in Japanese patients with CLD.

In this context, the aims of the present study were as follows: (1) to investigate the *in vivo* CYP2C19 activity in Japanese CLD patients with different CYP2C19 genotypes to assess the degree of interaction between CLD and genetic polymorphism on CYP2C19 activity, (2) to investigate which routine biochemical markers are clinically useful to predict CYP2C19 activity in CLD patients, and (3) to investigate the CLD-induced changes in CYP3A activity using the 3-hour partial formation clearance of cortisol to 6β-hydroxycortisol (6β-HC) as an in vivo CYP3A index.

MATERIALS AND METHODS

Thirty-one hepatitis C virus (HCV)–positive patients with chronic hepatitis or cirrhosis were studied. The diagnoses were established by histologic findings of liver biopsy and diagnostic imaging using computed tomography and ultrasonography. Table I shows the demographic and clinical characteristics of the 31 patients and the 30 healthy subjects who were enrolled in this study. The study protocol was approved by the Jikei University Ethics Committee. The nature and purpose of the study were fully explained to each subject before written informed consent was obtained.

Nine patients with chronic hepatitis (8 men and 1 woman) aged 52 to 84 years (mean ± SD, 66.0 ± 9.5 years) and weighing 40 to 74 kg (56.9 ± 10.2 kg), as well as 22 biopsy-proven cirrhotic patients (14 men and 8 women) aged 62 to 86 years (70.8 ± 6.1 years) and weighing 38 to 74 kg (55.1 ± 9.0 kg) were studied. The subjects were hospitalized at the Daisan Hospital of Jikei University School of Medicine. All patients were diagnosed as having chronic HCV infection by the detection of HCV-specific messenger RNA and antibody. One cirrhotic patient manifested ascites confirmed by abdominal ultrasonography and computed tomogra-

phy. Five cirrhotic patients had endoscopy-confirmed esophageal varices. The severity of liver impairment (cirrhosis) was classified according to the Child-Pugh classification.[14] Among the cirrhotic patients, 20 were graded as type A, 1 as type B, and 1 as type C. None had hepatic encephalopathy or had been treated with a transjugular intrahepatic portosystemic shunt[15] Their clinical and biochemical data are summarized in Table I. They were normotensive and had no abnormalities in 12-lead electrocardiogram (ECG) recordings. All patients had been hospitalized for at least 2 weeks before the study and had no drugs or foods that might have inhibited or induced CYP activity.

Thirty healthy men, aged 25 to 67 years (50.6 ± 16.4 years) and weighing between 32 and 86 kg (61.5 ± 12.7 kg), participated in the study. All subjects were confirmed to be in good general health based on a complete physical examination, a standard 12-lead ECG, hemogram, and clinical laboratory tests. They had no known histories of allergic reactions to drugs or gastrointestinal, renal, hepatic, pulmonary, cardiac, or hematologic disease; they took no drugs or foods that might have an influence on CYP activity.

After fasting overnight, healthy subjects and patients with CLD received 20 mg omeprazole with 200 mL of water, and blood samples were withdrawn at 1.5 hours after dosing to determine plasma cortisol concentration and at 3 hours after dosing to determine plasma concentrations of omeprazole and 5-hydroxyomeprazole. In addition, 3-hour timed urine samples were collected. DNA samples were extracted from the buffy coat of blood samples, according to the standard protocol described below. All samples were numbered and handled anonymously to ensure the participants' privacy. All enrolled subjects and patients were asked to refrain from smoking during the entire study period.

Genotyping

Seven milliliters of peripheral blood was obtained, and DNA was extracted from peripheral leukocytes using a DNA extraction kit (IsoQuick, Micro Probe Co, Garden Grove, Calif). Genotyping analysis of the polymorphic CYP2C19 genes was performed according to the methods of de Morais et al[12] and Kubota et al.[13] The CYP2C19*2 and CYP2C19*3 alleles were detected by polymerase chain reaction methods using the following allele-specific primers: for identifying the CYP2C19*2 allele, the forward primer was 5′-AATTACAACCAGAGCTTGGC-3′ and the reverse primer 5′-TATCACTTTCCATAAAAGCAAG-3′; for identifying the CYP2C19*3 allele, the forward primer was 5′-AACATCAGGATTGTAAGCAC-3′, and the reverse primer was 5′-TCAGGGCTTGGTCAATATAG-3′. Amplified fragments were digested with endonuclease Msp I (25 units) for CYP2C19*2 and Bam HI (25 unit) for CYP2C19*3 followed by electrophoresis in 3% agarose gels.

Phenotypes of CYP2C19 were predicted based on the band patterns of the polymerase chain reaction products, according to the method of Kubota et al.[13] Briefly, samples showing either CYP2C19*2 or CYP2C19*3 mutation in a homozygous state or CYP2C19*2 and CYP2C19*3 mutations in a combined heterozygous state were classified as the PM phenotype. Samples showing either CYP2C19*2 or CYP2C19*3 mutation in a heterozygous state were classified as the heterozygous EM phenotype, and those showing no CYP2C9*2 or CYP2C19*3 alleles were classified as the homozygous EM phenotype.[9,10]

Determination of Omeprazole and Its 5-Hydroxylated Metabolite in Plasma Samples

Omeprazole, the 5-hydroxy metabolite of omeprazole, and an internal standard [4,6-dimethyl-2-[(4-methoxy-2-pyridinyl)methyl]sulphinyl]-1*H*-benzimidazole] in plasma samples (500 µL) were extracted at pH 7.0 into ethyl acetate (1 mL).[16] A portion of the extract (150 µL) was injected onto a normal-phase liquid chromatographic column (LiChrospher Diol, 5 µm, 120 × 4.0 mm, Merck, Germany). The mobile phase consisting of 0.05% ammonium hydroxide, 0.8% water, 8% methanol, and 55% isohexane in ethyl acetate was delivered at a flow rate of 1.0 mL/min. Retention times were 3.5, 8.0, and 5.5 minutes for omeprazole, the 5-hydroxyomeprazole, and the internal standard, respectively. The analytic compounds in the eluate were detected using a UV absorption method at 302 nm. The absolute recovery of omeprazole was greater than 90% at 25 to 2500 nmol/L, and that for 5-hydroxy metabolite was 70% at 50 to 3000 nmol/L. The limit of quantification for omeprazole and 5-hydroxyomeprazole were 25 and 50 nmol/L, respectively, with coefficients of variation (CV) of less than 20%.

Determination Plasma Cortisol and Urinary 6β-Hydroxycortisol Levels

Urinary concentrations of unconjugated 6β-HC were assayed with a high-performance liquid chromatography (HPLC) coupled with an ultraviolet (UV) absorption method according to that of Bienvenu et al, with minor modifications.[17,18] Briefly, the mobile phase consisting of mixtures of acetonitrile/water/trichloroacetic acid (8/92/0.0005 vol/vol/wt, respectively) were ad-

justed to pH 2.5 by phosphoric acid and used in the assay. The HPLC system consisted of a reverse phase column (Prodigy 5μODS, 150 × 4.6 mm, Phenomenex, Torrance, Calif), a pump (L-7100, Hitachi Co Ltd, Tokyo, Japan), a UV detector (UV-8000, Tosoh Co Ltd, Tokyo, Japan) set at 244 nm, and a chromato-integrator (D-7500, Hitachi). The recovery rates for 6β-HC, and the internal standards extracted from urine samples were greater than 95% with CV below 4%. Within-day and between-day CV for determining 200 and 20 ng/mL of urinary 6β-HC and cortisol were both below 5%. Plasma cortisol concentrations were assayed using a fluorescence polarization immunoassay method (TDX system, Abbott Diagnostics, South Pasadena, Calif). Samples were prepared according to the manufacturer's instruction. Within-day and between-day CV for determining 0.05 and 0.1 μg/mL cortisol were both below 5%.

Pharmacokinetic and Statistical Analyses

Partial cortisol clearance to 6β-HC ($CL_{cortisol \rightarrow 6\beta\text{-}HC}$) was calculated as follows:

$$CL_{cortisol \rightarrow 6\beta\text{-}HC} = [Ae_{(6\beta\text{-}HC)}/3]/C_{cortisol} \quad \text{(Equation 1)}$$

where $Ae_{(6\beta\text{-}HC)}/3$ represents the average urinary excretion rates of 6β-HC during the 3-hour urine collection period, and $C_{cortisol}$ is the plasma cortisol concentration determined at the midpoint of the urine collection period.

Equation 1 is based on the assumption that the rate of 6β-HC formation from cortisol equals the rate of appearance in urine and that 6β-HC is eliminated in the urine without further metabolism. In addition, Equation 1 is based on the assumption that the midpoint plasma cortisol concentration represents the mean plasma cortisol concentration during the urine sampling period and that collection of urine is complete. Based on these assumptions, we consider that $CL_{cortisol \rightarrow 6\beta\text{-}HC}$ would represent the enzyme activity involved in the formation of 6β-HC from cortisol. This parameter has been reported and assessed previously by our group using a potent CYP3A inhibitor, clarithromycin.[18]

Data are expressed as mean ± SEM throughout the study, unless otherwise stated. The mean values for the omeprazole hydroxylation index and $CL_{cortisol \rightarrow 6\beta\text{-}HC}$ between the CLD group and the control group as well as within the same group were compared statistically by multiple comparisons analysis followed by Dunnett's *t* test. Correlation between the above 2 indices of the *in vivo* metabolic activities of CYP2C19 and CYP3A and clinical characteristics and/or biochemical parameters was analyzed by multiple regression analysis (SAS version 8.2, SAS Institute, Cary, NC). A *P* value of <.05 was considered statistically significant.

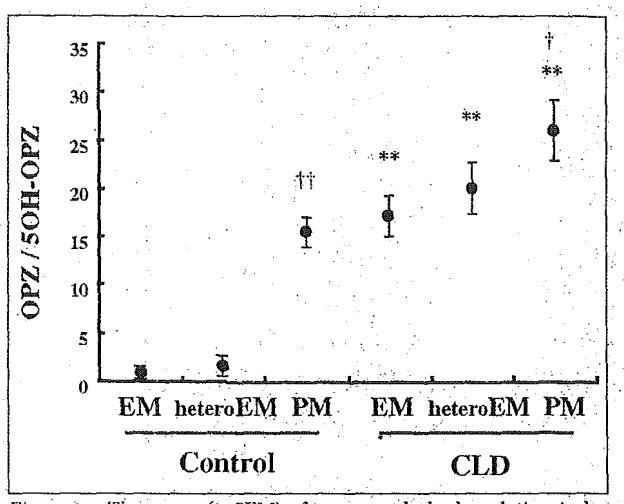

*Figure 1. The mean (± SEM) of omeprazole hydroxylation index (OPZ/5OH-OPZ) in healthy subjects and patients with chronic liver disease having the respective genotypes of CYP2C19. EM = extensive metabolizers with the homozygous wild-type CYP2C19 genotype; heteroEM = heterozygous extensive metabolizer with the heterozygous CYP2C19*2 or CYP2C19*3 genotype (CYP2C19*1/*2 or *1/*3); PM = poor metabolizers with homozygous CYP2C19*2 and CYP2C19*3 (CYP2C19*2/*2 or *3-*3) or combined heterozygous CYP2C19*2 and CYP2C19*3 genotypes (CYP2C19*2/*3); OPZ/5OH-OPZ = plasma omeprazole to 5-hydroxyomeprazole ratio; CLD = chronic liver diseases (chronic hepatitis and cirrhosis). **P < .01 compared with the control subjects having the corresponding genotypes of CYP2C19. ††P < .001 compared with other genotypes (EM, heteroEM) of the control group. †P < .05 compared with EM patients with chronic liver disease.*

RESULTS

Omeprazole to 5-Hydroxyomeprazole Ratio in the Controls and Patients With CLD

The 3-hour omeprazole hydroxylation index (ie, 3-hour postdose plasma concentration ratio of omeprazole/5-hydroxyomeprazole [OPZ/5OH-OPZ] ratio) has been considered to be a useful *in vivo* biomarker of CYP2C19 activity by many investigators.[19-22] Because the index has an inverse relationship with CYP2C19 activity, a higher index indicates lower *in vivo* CYP2C19 activity.

In healthy subjects with different CYP2C19 genotypes, the mean OPZ/5OH-OPZ ratio was significantly ($P < .01$) higher in subjects with the PM genotypes (15.5 ± 1.52) than in those with homozygous EM (0.81 ± 0.09) and heterozygous EM (1.55 ± 0.20) genotypes (Figure 1). In patients with CLD, the mean OPZ/5OH-OPZ ratio

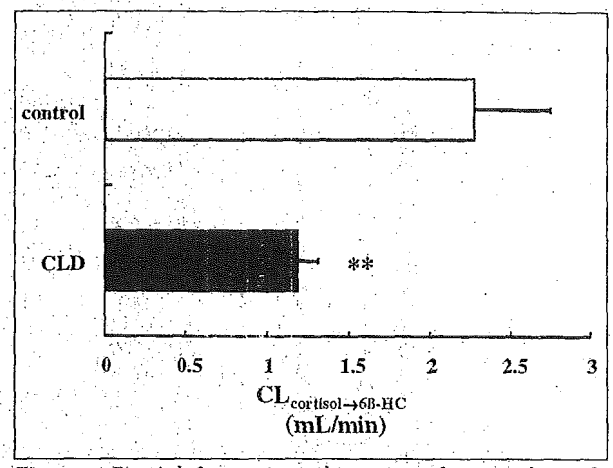

*Figure 2. Partial formation clearance of cortisol to 6β-hydroxycortisol ($CL_{cortisol \rightarrow 6\beta\text{-}HC}$) in patients with chronic liver disease and in control subjects. $CL_{cortisol \rightarrow 6\beta\text{-}HC}$ = partial formation clearance of cortisol to 6β-hydroxycortisol; CLD = chronic liver disease. **P < .001 compared with the control group.*

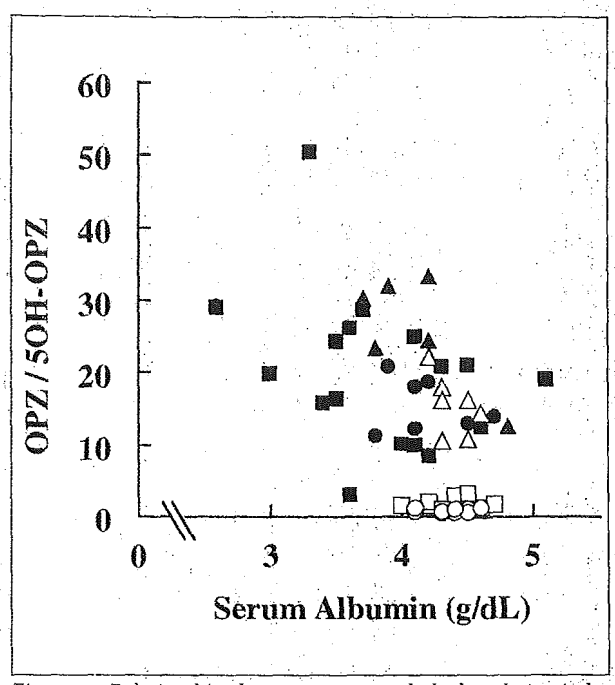

Figure 3. Relationships between omeprazole hydroxylation index (OPZ/5OH-OPZ) and serum concentrations of albumin in patients with chronic liver diseases (CLD) and the healthy control subjects having different CYP2C19 genotypes. The CLD patients with the CYP2C19 genotypes of homozygous extended metabolizer, heterozygous extended metabolizer, and poor metabolizer are denoted by closed circles (●), squares (■), and triangles(▲), respectively, and the healthy subjects having the corresponding CYP2C19 genotypes are denoted by open circles (○), squares (□), and triangles (△), respectively. The relationship between serum albumin level and hydroxylation index in CLD patients and healthy subjects is statistically significant (n = 61, r = −.526, P < .001, Y = 63.5 − 12.3X). OPZ/5OH-OPZ = plasma omeprazole to 5-hydroxyomeprazole concentration ratio.

was significantly (P < .05) greater in patients with the PM genotypes (26.04 ± 3.15; n = 6) than in those with the homozygous EM genotype (17.15 ± 2.12; n = 7), and the ratio in those with heterozygous EM genotypes (*1/*2, n = 11; *1/*3, n = 6; 20.02 ± 2.63) was in between the values of the 2 homozygous genotypes. The omeprazole hydroxylation index of patients with different CYP2C19 genotypes was significantly (P < .01) greater than that of control subjects with the corresponding genotypes.

Partial Formation Clearance of 6β-Hydroxycortisol ($CL_{cortisol \rightarrow 6\beta\text{-}HC}$)

The mean value of $CL_{cortisol \rightarrow 6\beta\text{-}HC}$ obtained from CLD patients was significantly ($P < .001$) lower than that obtained from the control group (1.19 ± 0.12 versus 2.26 ± 0.24 mL/min) (Figure 2). In patients with CLD, the mean $CL_{cortisol \rightarrow 6\beta\text{-}HC}$ decreased with an increase in severity of liver impairments as follows: 1.35 ± 0.26 in patients with chronic hepatitis and 1.12 ± 0.13 in patients with cirrhosis. The value was significantly lower ($P < .05$) in patients with cirrhosis compared with control subjects.

Correlations Between Omeprazole Hydroxylation Index or $CL_{cortisol \rightarrow 6\beta\text{-}HC}$ and Patients' Covariates

Table 2 shows the results of multiple regression analysis between the omeprazole hydroxylation index or $CL_{cortisol \rightarrow 6\beta\text{-}HC}$ and patients' covariates (ie, demographic parameters and laboratory tests) in CLD patients and control subjects. A significant ($P < .05$) correlation was observed between omeprazole hydroxylation index and disease condition, serum albumin level, or CYP2C19 genotype (Table 2). On the other hand, no significant correlation was observed between $CL_{cortisol \rightarrow 6\beta\text{-}HC}$ and all the variables tested, except disease condition (Table 2). There was a significant relationship between serum albumin concentration and the omeprazole hydroxylation index for overall data (n = 61, $r = .526$, $P < .001$) (Figure 3). When we analyzed the subset of CLD patients with the homozygous and heterozygous EM genotypes of CYP2C19 (ie, excluding control subjects and CLD patients with the PM genotype), the relation between serum albumin concentration and omeprazole hydroxylation index remained significant (n = 25, $r = -.428$, $P < .05$).

Table II Multiple Regression Analysis on the Relationships Between the *in Vivo* Indices of CYP2C19 (Omeprazole Hydroxylation Index) or CYP3A Activity ($CL_{cortisol \rightarrow 6\beta\text{-}HC}$) and Patients' Covariates in Patients With Chronic Liver Diseases and Healthy Controls

	Versus Omeprazole Hydroxylation Index n = 61 R^2 = .7300			Versus $CL_{cortisol \rightarrow 6\beta\text{-}HC}$ n = 61 R^2 = 3088		
Variable	Partial Regression Coefficient	*P*	Standardized Partial Regression Coefficient	Partial Regression Coefficient	*P*	Standardized Partial Regression Coefficient
Disease condition[a]	13.792	<.0001	0.612	−0.956	.0491	−0.420
Age	0.014	.8377	0.020	−0.012	.2761	−0.169
Sex	0.031	.9869	0.001	−0.277	.3673	−0.116
Albumin	−8.026	.0095	−0.344	−0.501	.3022	−0.213
Total bilirubin	0.641	.2718	0.115	−0.007	.9401	−0.013
AST	−0.090	.3175	−0.268	−0.013	.3589	−0.393
ALT	0.052	.3862	0.162	0.011	.2772	0.326
Serum creatinine	−2.870	.4739	−0.064	−0.405	.5310	−0.090
CYP2C19 genotype[b]	11.097	<.0001	0.403	0.083	.8171	0.030

AST, asparate aminotransferase; ALT, alanine aminotransferase.
a. Disease condition implies patients with chronic hepatitis (n = 9), patients with liver cirrhosis (n = 22), and healthy controls.
b. CYP2C19 genotype implies 3 genotypes: homozygous extensive, heterozygous extensive, and poor metabolizer.

DISCUSSION

The present study is the first to investigate the influence of CLD on the *in vivo* CYP2C19 activity in patients having different genotypes of CYP2C19 using the 3-hour omeprazole hydroxylation index (ie, 3-hour postdose plasma OPZ/5OH-OPZ concentration ratio). We demonstrated a dramatic reduction in this biomarker of *in vivo* CYP2C19 activity in CLD patients with the homozygous and heterozygous EM genotypes to the extent that the activity became comparable to the level of healthy subjects with the PM genotype (Figure 1). Because subjects with the PM genotypes for CYP2C19 have no functional CYP2C19 enzymes, our findings are compatible with the notion that the expression of CYP2C19 in the homozygous or heterozygous EM patients with CLD should have been suppressed to an almost null level. Because CYP2C19 is involved in the hepatic metabolism of many therapeutically important drugs other than omeprazole, patients with CLD may be susceptible to adverse reactions from taking drugs that depend on CYP2C19 activity for metabolism. In addition, CLD patients with the PM genotype had a significantly ($P < .05$) higher hydroxylation index than those with the homozygous EM genotype. Because the omeprazole hydroxylation index has a reciprocal relationship with the *in vivo* CYP2C19 activity, whether this statistically significant difference is associated with a clinically relevant difference in dose requirement remains to be studied.

Our data show a good agreement with the previous studies in patients with CLD[1,2] that demonstrated a preferential reduction (77% from the baseline) of CYP2C19 activity assessed by the hydroxylation of mephenytoin compared with that of CYP3A4 (28%) and CYP2D6 (4%) assessed by hydroxylation of dapsone and debrisoquine, respectively. In the present study, the hydroxylation index of omeprazole in CLD patients with the homozygous EM genotype increased more than 10-fold than that of the healthy subjects with the same genotype, indicating a marked reduction in CYP2C19 activity associated with CLD. It is of note that the majority of our CLD patients had only mild liver dysfunction (9 had chronic hepatitis, and 20 of 22 cirrhotic patients were Child-Pugh type A), indicating that compared with other CYP isoforms (eg, CYP3A4), the hepatic CYP2C19 activity is more susceptible to CLD. This degree of reduction is in agreement with the report of Andersson et al.[23] In contrast, the index of *in vivo* CYP3A activity, $CL_{cortisol \rightarrow 6\beta\text{-}HC}$, obtained from CLD patients decreased to only a half of the control value.

Kimura et al[24] studied the omeprazole hydroxylation index in Japanese healthy subjects and patients with gastrointestinal disease and found that the hy-

droxylation indices obtained from 3 CLD patients with CYP2C19*1/*1 (n = 1) or CYP2C19*1/*2 (n = 2) were comparable with those observed in their healthy controls with the PM genotypes. Because they studied only 3 CLD patients under multiple dosing of the drug, they were unable to conclude whether their finding was attributable to a CLD-induced reduction of CYP2C19 activity or to partial saturation of the enzyme by multiple dosing. Our data confirm that the dramatic increase of the omeprazole hydroxylation index in CLD patients does represent a substantial reduction in the CYP2C19 activity in CLD patients. Nevertheless, the precise mechanism associated with the preferential reduction of this CYP isoform remains obscure at present.

We found that the omeprazole hydroxylation index obtained from CLD patients with the PM genotypes was further increased as compared with healthy subjects having the same genotype. Our data suggest that hepatic drug metabolizing enzyme(s) other than CYP2C19 may be involved in the *in vivo* 5-hydroxylation of omeprazole because both groups should have had no functional CYP2C19 enzyme. Our data are consistent with the study of Furuta et al,[10] demonstrating that the area under the plasma concentrations (AUC) ratio of OPZ/5OH-OPZ in healthy subjects with PM genotypes coadministered the CYP3A4 inhibitor, clarithromycin, was 4 times greater than the value obtained from the same subjects given omeprazole alone. In the present study, we observed that the mean value of a putative index of CYP3A activity, $CL_{cortisol \rightarrow 6\beta\text{-}HC}$, was approximately 50% lower in CLD patients than in control subjects. The data of Furuta et al[10] and of our study collectively suggest that CYP3A4 may be involved in part in the 5-hydroxylation of omeprazole.

There are certain limitations in the present study. We assigned the phenotypes of CYP2C19 based solely on the genotyping of the 2 major mutations (CYP2C19*2 in exon 5 and CYP2C19*3 in exon 4). We did not genotype other rare variant alleles (eg, from CYP2C19*4 to CYP2C19*12[25,26]) that have been reported in non-Asian populations. However, because these 2 variant alleles have been shown to account for 98% of the PM phenotype in the Japanese population,[6,7] we consider our assignment of the CYP2C19 phenotype to be valid. In addition, we evaluated the in vivo CYP2C19 activity based on the plasma omeprazole hydroxylation index at 3-hour postdose after a single oral load of 20 mg. Therefore, we cannot totally deny the possibility that an interindividual variability in the intestinal drug absorption may have jeopardized an accurate assessment of the enzyme activity. However, Andersson et al[23] and Renetti et al[27] reported that time required to reach peak plasma concentration of the drug was less than 3 hours after oral administration. We therefore consider that the 3-hour postdose hydroxylation index would be a robust *in vivo* index of CYP2C19 activity.

On the other hand, omeprazole is also known to be metabolized to omeprazole sulfone *via* CYP3A, and omeprazole sulfone is further metabolized to 5-hydroxyomeprazole sulfone via CYP2C19.[28,29] In PMs of CYP2C19, the metabolic pathway of omeprazole to omeprazole sulfone is supposed to be enhanced, resulting in an accumulation of the sulfone metabolite. However, Ieiri et al[22] have demonstrated that PM status does not elevate the omeprazole to omeprazole sulfone ratio (1.8 to 2.5–3; not significant) or change the AUC ratio (1.8 to 0.8–0.9) compared with the EM status. Their results suggest that the change in omeprazole hydroxylation index is mostly because of a change in CYP2C19 activity, with a minor contribution from CYP3A4, even in those with PM status. Because we did not measure the sulfone level, it remains unknown how liver damage modifies the metabolism of omeprazole in PMs and heterozygous EMs.

Many factors including gender,[30,31] concomitant medication,[24,28,29] age,[32] liver disease,[23,24,27] and length of the therapy[33] have been reported to affect the *in vivo* metabolic activity of CYP2C19, thus confounding a simple relationship between genotypes and phenotypes. The multiple regression analysis performed in the present study revealed that age and gender were not associated with the reduction in *in vivo* CYP2C19 activity. However, caution has to be exercised in interpreting these results because the mean age of CLD patients was significantly higher than that of healthy controls (Table 1) and the relationship between age and CYP2C19 activity might not have been linear as assumed by the analysis. Several reports[34-36] have demonstrated a significant correlation between the *in vivo* CYP2C19 activity and certain biochemical parameters associated with liver function (albumin, transaminase, and prothrombin index). The multiple regression analysis in the present study revealed that only serum albumin level, disease condition (ie, presence or absence of CLD), and genotype may explain the decrease of *in vivo* CYP2C19 activity.

We found that the mean $CL_{cortisol \rightarrow 6\beta\text{-}HC}$ obtained from CLD patients was decreased to 48% of that obtained from the healthy subjects. Because CYP3A isoforms (eg, CYP3A4 and CYP3A5) are primarily responsible for 6β-hydroxylation of the endogenous cortisol, $CL_{cortisol \rightarrow 6\beta\text{-}HC}$ may be a clinically useful tool as an *in vivo*

parameter of CYP3A activity. We have already reported that a 3-hour $CL_{cortisol \rightarrow 6\beta\text{-}HC}$ is a better clinical index of in vivo CYP3A activity than is the traditionally employed ratio of urinary 6β-HC to cortisol for the assessment of the inhibitory effect of clarithromycin on CYP3A activity in patients receiving *Helicobacter pylori* eradication therapy.[18] An oral administration of clarithromycin at 800 mg/d reduced $CL_{cortisol \rightarrow 6\beta\text{-}HC}$ to approximately 50% of the baseline value. The present finding that the mean $CL_{cortisol \rightarrow 6\beta\text{-}HC}$ of CLD patients was reduced by approximately 50% compared with healthy controls implies that the magnitude of reduction in *in vivo* CYP3A activity induced by CLD is largely comparable to that induced by clarithromycin. However, multiple regression analysis detected no significant correlation between $CL_{cortisol \rightarrow 6\beta\text{-}HC}$ and biochemical parameters associated with liver function. Thus, there is controversy of whether the 6β-hydroxylation of cortisol is a reliable index of the *in vivo* hepatic CYP3A activity. It has been suggested that a certain amount of cortisol is secreted into the gut and is metabolized by the epithelial CYP3A on reabsorption. It is possible that CYP3A5 is expressed in the kidneys. Thus, the overall catalytic activity of cortisol 6β-hydroxylation may be attributed not only to the hepatic CYP3A activity but also to certain extrahepatic tissues (eg, the kidney and the small intestine).[37,38] This may be a reason we did not obtain any significant correlation between $CL_{cortisol \rightarrow 6\beta\text{-}HC}$ and the biochemical parameters of hepatic function. In addition, there is a concern that omeprazole administered to CLD patients and control subjects might have affected the CYP3A4 activity measured by $CL_{cortisol \rightarrow 6\beta\text{-}HC}$. However, Furuta et al[39] reported in their *in vitro* study performed with human liver microsomes that omeprazole is 50 times weaker as an inhibitor for CYP3A4 than for CYP2C19, and Tateishi et al[40] demonstrated that omeprazole did not affect *in vivo* erythromycin breath test, an established index of CYP3A activity, in healthy subjects. In this context, we consider that the administration of omeprazole did not interfere with $CL_{cortisol \rightarrow 6\beta\text{-}HC}$.

In conclusion, the present study demonstrated that the impaired CYP2C19 and CYP3A activity in CLD patients may be reflected by an increase in the omeprazole hydroxylation index and a decrease in $CL_{cortisol \rightarrow 6\beta\text{-}HC}$, respectively. It is interesting that CLD appears to induce preferential reduction in CYP2C19 activity compared with CYP3A. The omeprazole hydroxylation index of CLD patients with the EM genotype of CYP2C19 was largely comparable to that of control subjects with the PM genotype. Further studies are required to assess whether the *in vivo* index of CYP2C19 may be useful for dosage adjustment of drugs that are eliminated mainly via CYP2C19-mediated hepatic metabolism.

REFERENCES

1. Morgan DJ, McLean AJ. Clinical pharmacokinetic and pharmacodynamic considerations in patients with liver disease: an update. *Clin Pharmacokinet.* 1995;29:370-391.

2. Adedoyin A, Arns PA, Richards WO, Wilkinson GR, Branch RA. Selective effect of liver disease on the activities of specific metabolizing enzymes: investigation of cytochromes P450 2C19 and 2D6. *Clin Pharmacol Ther.* 1998;64:8-17.

3. May G, Arns PA, Richards WO, et al. The disposition of dapsone in cirrhosis. *Clin Pharmacol Ther.* 1992;51:689-700.

4. Lown K, Kolars J, Turgeon K, Merion R, Wrighton SA, Watkins PB. The erythromycin breath test selectively measures P450IIIA in patients with severe liver disease. *Clin Pharmacol Ther.* 1992;51:229-238.

5. Guengerich FP, Turvy CG. Comparison of levels of several human microsomal cytochrome P-450 enzymes and epoxide hydrolase in normal and disease states using immunochemical analysis of surgical liver samples. *J Pharmacol Exp Ther.* 1991;256:1189-1194.

6. George J, Liddle C, Murray M, Byth K, Farrell GC. Pre-translational regulation of cytochrome P450 genes is responsible for disease-specific changes of individual P450 enzymes among patients with cirrhosis. *Biochem Pharmacol.* 1995;49:873-881.

7. George J, Murray M, Byth K, Farrell GC. Differential alterations of cytochrome P450 proteins in livers from patients with severe chronic liver disease. *Hepatology.* 1995;21:120-128.

8. Weinshilboum R. Inheritance and drug response. *New Engl J Med.* 2003;348:529-537.

9. Desta Z, Zhao X, Shin J-G, Flockhart DA. Clinical significance of the cytochrome P450 2C19 genetic polymorphism. *Clin Pharmacokinet.* 2002;41:913-958.

10. Furuta T, Ohashi K, Kobayashi K, et al. Effects of clarithromycin on the metabolism of omeprazole in relation to CYP2C19 genotype status in human. *Clin Pharmacol Ther.* 1999;66:265-274.

11. Furuta T, Shirai N, Watanabe F, et al. Effect of cytochrome P4502C19 genotypic differences on cure rates for gastroesophageal reflux disease by lansoprazole. *Clin Pharmacol Ther.* 2002;721:453-460.

12. de Morais SMF, Wilkinson GR, Blaisdell J, Meyer UA, Nakamura K, Goldstein JA. Identification of a new genetic defect responsible for the polymorphism of (S)-mephenytoin metabolism in Japanese. *Mol Pharmacol.* 1994;46:594-598.

13. Kubota T, Chiba K, Ishizaki T. Genotyping of S-mephenytoin 4'-hydroxylation in an extended Japanese population. *Clin Pharmacol Ther.* 1996;60:661-666.

14. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding varices. *Br J Surg.* 1973; 60:646-649.

15. Chalasani N, Gorski JC, Patel NH, Hall SD, Galinsky RE. Hepatic and intestinal cytochrome P450 3A activity in cirrhosis: effects of transjugular intrahepatic portosystemic shunt. *Hepatology.* 2001; 34:1103-1108.

16. Andersson T, Lagerstrm P-O. High-performance liquid chromatographic assay for human liver microsomal omeprazole metabolism. *J Chromaogr.* 1993;619:291-297.

17. Bienvenu T, Rey E, Pons G, d'Athis P, Olive G. A simple non-invasive procedure for the investigation of cytochrome P-450IIIA dependent enzymes in human. *Int J Clin Pharmacol Ther Toxicol.* 1991; 29:441-445.

18. Ushiama H, Echizen H, Nachi S, Ohnishi A. Dose-dependent inhibition of CYP3A activity by clarithromycin during *Helicobacter pylori* eradication therapy assessed by changes in plasma lansoprazole levels and partial cortisol clearance to 6β-hydroxycortisol. *Clin Pharmacol Ther.* 2002;72:33-43.

19. Chang M, Dahl M-L, Tybring G, Gotharson E, Bertilsson L. Use of omeprazole as a probe drug for CYP2C19 phenotype in Swedish Caucasians: comparison with S-mephenytoin hydroxylation phenotype and CYP2C19 genotype. *Pharmacogenetics.* 1995;5:358-363.

20. Chang M, Tybring G, Dahl M-L, et al. Interphenotype differences in disposition and effect on gastrin levels of omeprazole—suitability omeprazole as a probe for CYP2C19. *Br J Clin Pharmacol.* 1995;39: 511-518.

21. Roh H-K, Dahl M-L, Tybring G, Yamada H, Cha Y-N, Bertilsson L. CYP2C19 genotype and phenotype determined by omeprazole 8 in Korean population. *harmacogenetics.* 1996;6:547-551.

22. Ieiri I, Kubota Y, Urae A, et al. Pharmacokinetics of omeprazole (a substrate of $CYP2C19_{m1}$ in exon 5 and $CYP2C19_{m2}$ in exon 4, in Japanese subjects. *Clin Pharmacol Ther.* 1996;59:647-653.

23. Andersson T, Olsson R, Regårdh C-G, Skånberg I. Pharmacokinetics of [^{14}C]omeprazole in patients with liver cirrhosis. *Clin Pharmacokinet.* 1993;24:71-78.

24. Kimura M, Ieiri I, Wada Y, et al. Reliability of the omeprazole hydroxylation index for CYP2C19 phenotyping: possible effect of age, liver disease and length of therapy. *Br J Clin Pharmaco.* 1999;47: 115-119.

25. Ferguson RJ, De Morais SM, Benhamou S, et al. A new genetic defect in human CYP2C19: mutation of the initiation codon is responsible for poor metabolism of S-mephenytoin. *J Pharmacol Exp Ther.* 1998;284:356-361.

26. Blaisdell J, Mohrenweiser H, Jackson J, et al. Identification and functional characterization of new potentially defective alleles of human CYP2C19. *Pharmacogenetics.* 2002;12:703-711.

27. Rinetti M, Regazzi MB, Villanti P, Tizzoni M, Sivelli R. Pharmacokinetics of omeprazole in cirrhotic patients. *Arzneimittelforschung.* 1991;41:420-422.

28. Andersson T. Omeprazole drug interaction studies. *Clin Pharmacokinet.* 1991;21:195-212.

29. Andersson T. Pharmacokinetics, metabolism and interactions of acid pump inhibitors. *Clin Pharmacokinet.* 1996;36:9-28.

30. Xie H-G, Huang S-L, Xu Z-H, Xiao Z-S, He N, Zhou H-H. Evidence for the effect of gender on activity of (S)-mephenytoin 4'-hydroxylase (CYP2C19) in a Chinese population. *Pharmacogenetics.* 1997;7:115-119.

31. Kim M-J, Bertino JS Jr, Gaedigk A, Zhang Y, Sellers EM, Nafziger AN. Effect of sex and menstrual cycle phase on cytochrome P450 2C19 activity with omeprazole used as a biomaker. *Clin Pharmacol Ther.* 2002;72:192-199.

32. Landahl S, Andersson T, Larsson M, et al. Pharmacokinetic study of omeprazole in elderly healthy volunteers. *Clin Pharmacokinet.* 1992;23:469-476.

33. Chang M, Tybring G, Dahl M-L, et al. Interphenotype differences in disposition and effect on gastrin levels of omeprazole—suitability of omeprazole as a probe for CYP2C19. *Br J Clin Pharmacol.* 1995; 39:511-518.

34. Bergquist C, Lindegård J, Salmonson T. Dosing recommendations in liver disease. *Clin Pharmacol Ther.* 1999;66:201-204.

35. Rodighiero V. Effects of liver disease on pharmacokinetics: an update. *Clin Pharmacokinet.* 1999;37:399-431.

36. Veromese L, Rautaureau J, Sadler BM, et al. Single-dose pharmacokinetics of amprenavir, a human immunodeficiency virus type 1 protease inhibitor, in subjects with normal or impaired hepatic function. *Antimicrob Agents Chemother.* 2000;44:821-826.

37. Seidegard J, Dahlstrm K, Kullberg A. Effect of grapefruit juice on urinary 6β-hydroxycortisol/cortisol excretion. *Clin Exp Pharmacol Physiol.* 1998;25:379-381.

38. Schuetz EG, Schuetz JD, Grogan WM, et al. Expression of cytochrome P4503A in amphibian, rat and human kidney. *Arch Biochem Biophys.* 1992;294:206-214.

39. Furuta S, Kamada E, Suzuki T, et al. Inhibition of drug metabolism in human liver microsomes by nizatidine, cimetidine and omeprazole. *Xenobiotica.* 2001;31:1-10.

40. Tateishi T, Graham SG, Krivoruk Y, Wood AJJ. Omeprazole does not affect measured CYP3A4 activity using the erythromycin breath test. *Br J Clin Pharmacol.* 1995;40:411-412.