Different Contribution of CYP2C19 in the *in Vitro* Metabolism of Three Proton Pump Inhibitors

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A series of clinical studies on the *cytochrome P450 2C19* (*CYP2C19*) genotype and the pharmacokinetics and pharmacodynamics of three proton pump inhibitors (PPIs), omeprazole, lansoprazole and rabeprazole, have been conducted to establish the individualized pharmacotherapy based on the *CYP2C19* genotyping, and in the present study, the *CYP2C19* genotype-dependency was more pronounced for omeprazole than the other two. Herein, to validate further the difference among 3 PPIs in *CYP2C19* genotype-dependency on the phenotype, a comparative *in vitro* study was conducted using the human liver microsomes and newly developed anti-human CYP antibodies. The residual concentrations of omeprazole and lansoprazole in 5 lots of human liver microsomes were dependent on the CYP2C19 activities, however, for rabeprazole, there was no correlation. The hydroxylation of omeprazole was more inhibited by anti-CYP2C19 antibody than lansoprazole, whereas anti-CYP3A4 antibody showed similar inhibition. In conclusion, the relative contribution of CYP2C19 on total metabolism of 3 PPIs elucidated herein coincided with the *CYP2C19* genotype-dependent pharmacokinetics.

Key words human liver microsome; omeprazole; lansoprazole; rabeprazole; P450 activity; anti-human CYP antibody

Inter-individual variation in the pharmacokinetics and thereby pharmacodynamics of many therapeutic agents are possibly caused by genetic polymorphisms of drug metabolizing enzymes including cytochrome P450 2C9 (CYP2C9), CYP2C19 and CYP2D6. A series of clinical studies on the *CYP2C19* genotype and the pharmacokinetics and pharmacodynamics of three proton pump inhibitors (PPIs), omeprazole, lansoprazole and rabeprazole, were conducted to establish the individualized pharmacotherapy based on the *CYP2C19* genotyping.[1—5] The subjects were classified into homo extensive metabolizers, hetero extensive metabolizers and poor metabolizers according to the *CYP2C19* genotype diagnosed. The findings were summarized as follows: 1) plasma concentrations of omeprazole and lansoprazole after single oral administration were defined by the *CYP2C19* genotype, whereas rabeprazole was not, 2) the *CYP2C19* genotype-dependency of lansoprazole was weaker than omeprazole, and 3) hetero extensive metabolizers could be included with homo extensive metabolizers to be extensive metabolizers. There were several *in vitro* studies reporting that CYP2C19 and CYP3A4 were responsible for the metabolism of 3 PPIs, however, little information is available for their relative contributions on total metabolism,[6—10] that would be adequate to explain that the *CYP2C19* genotype-dependent pharmacokinetics was more marked for omeprazole than the other two. Herein, to validate further the difference among 3 PPIs in *CYP2C19* genotype-dependency on the phenotype, a comparative *in vitro* study on the metabolism of 3 PPIs was conducted using human liver microsomes and newly developed anti-human CYP antibodies.

MATERIALS AND METHODS

Materials Omeprazole and its two primary metabolites, 5-hydroxyomeprazole and omeprazole sulfone, were obtained from AstraZeneca Ltd. (Osaka, Japan). Lansoprazole and its two primary metabolites, 5-hydroxylansoprazole and lansoprazole sulfone, were obtained from Takeda Pharmaceutical Co. (Osaka, Japan). The internal standard for rabeprazole (IS735), rabeprazole and its two primary metabolites, thioether rabeprazole and rabeprazole sulfone, were obtained from Eisai Co. (Tokyo, Japan). All other chemicals were of reagent grade and obtained commercially. Six lots of human liver microsomes (Table 1) were purchased from KAC Co. Ltd. (Kyoto, Japan). Anti-human CYP antibodies raised against bacterial expressed recombinant human CYP2C19 and CYP3A4 (referred to as anti-CYP2C19 and anti-CYP3A4 antibodies, respectively) were prepared in rabbits according to the method of Kaminsky *et al.*[11] Cross reactivity of antibodies raised against CYP2C19 and 3A4 were checked by means of ELISA. Anti-CYP2C19 antibody recognized CYP2C19 and slightly cross reacted with CYP2C9, but did not react with CYP1A2, 2D6, 2E1 and 3A4. Under the condition employed in the present study, cross reaction between CYP2C9 and anti-CYP2C19 antibody is minimized. On the other hand, anti-CYP3A4 antibody recognized CYP3A4, but did not react with CYP1A2, 2C9, 2C19, 2D6 and 2E1 (data not shown).

***In Vitro* Metabolism of Omeprazole, Lansoprazole and Rabeprazole** Either of omeprazole, lansoprazole or rabeprazole (0.4 μM) was incubated in the reaction buffer consisting of 10 mM of HEPES (pH 7.4), 2 mM of $MgCl_2$, 0.02 mM of EDTA 2Na and 0.22—0.26 mg/ml of either lot of microsomes (Lot No. HHM-0230, HHM-0232, HHM-0233, HHM-0235 or HHM-0259). After the pre-incubation for 3 min at 37 °C, the reaction was initiated by the addition of 1 mM NADPH. The reaction was terminated at 30 min by the addition of 100 μl diethyl ether/methylene chloride (7/3

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Table 1. Characterization of Specific CYP450 Isozyme Activities in Human Liver Microsomes

		Lot# (HHM-) 0230	0232	0233	0235	0259	0264
	Gender / Age	Female 51 years	Female 29 years	Female 47 years	Male 19 years	10 Males Pool	10 Females Pool
P450 isozyme	Marker activity[a)]						
CYP1A2	Phenacetin *O*-deethylation	291	481	211	143	246	244
CYP2A6	Coumarin 7-hydroxylation	0.20	0.98	1.20	0.46	0.79	0.76
CYP2C19	Mephenytoin 4′-hydroxylation	59	160	3	4	16	43
CYP2D6	Dextromethorphan *O*-demethylation	52	87	24	40	114	70
CYP2E1	Chlorzoxazone 6-hydroxylation	926	763	1253	413	1133	974
CYP3A4	Testosterone 6β-hydroxylation	2.1	3.7	4.4	0.1	2.1	2.1
CYP4A	Lauric acid 12-hydroxylation	1.6	2.0	1.4	0.5	1.6	1.5

a) nmol/mg/min for CYP2A6, CYP3A4 and CYP4A; pmol/mg/min for others.

(v/v)) on ice. The NADPH was not added for the blank incubations. After terminating the reaction, 10 μl of each internal standard in methanol (0.1 mg/ml phenacetin, 2.5 μg/ml isobutyl-4-hydroxybenzoate or 0.1 mg/ml IS735) was added for each sample of omeprazole, lansoprazole or rabeprazole, respectively. Then, extraction was performed on a vortex mixer for 1 min followed by centrifugation for 10 min at 3000 rpm. An aliquot of the organic phase was evaporated to dryness under nitrogen at 40 °C, the residue was reconstituted in 120 μl of each mobile phase. After filtration with a 0.20 μm Milex-LG filter (Nihon Millipore, Osaka, Japan), each 30 μl aliquot was injected into the HPLC system.

Inhibition of PPI Metabolism by Anti-CYP2C19 and Anti-CYP3A4 Antibodies Each 5 or 10 μl of anti-CYP2C19 antibody (140.0 mg/ml), anti-CYP3A4 antibody (142.7 mg/ml) or rabbit pre-immune sera (142.6 mg/ml) as a control was added to the reaction buffer consisting of 10 mM of HEPES (pH 7.4), 2 mM of $MgCl_2$, 0.02 mM of EDTA 2Na and 0.22 mg/ml of Lot No. HHM-0264. After standing for 30 min at room temperature, each sample was pre-incubated for 3 min at 37 °C with the final concentration of each 5 μM PPI, followed by the same procedure related above. Compared with other *in vitro* experiments,[6,7,9,12,13] each PPI concentration in microsomal samples was lower (0.4 or 5 μM), and their values were close to or less than the maximum concentration in clinical use, that is, omeprazole: 1.5—3 μM, lansoprazole: 1.2—4 μM and rabeprazole: 1.2 μM.[1)]

HPLC Assay The method of HPLC assay for omeprazole, lansoprazole and rabeprazole and their metabolites was described previously.[1,3,4)] The HPLC system consisted of an LC-10AT pump, an SIL-10A auto-injector, an SPD-10A detector, a CTO-10A column oven (at 40 °C), an SCL-10A system controller, and a C-R7A chromatopack (Shimadzu Co., Kyoto, Japan).

Data Processing and Statistical Analysis Each experiment was conducted in triplicate. The relationship between the mean concentrations of omeprazole, lansoprazole and rabeprazole and their metabolites and the CYP2C19 or CYP3A4 activity was analyzed by means of the least squares method. The effects of anti-CYP2C19 and anti-CYP3A4 antibodies were evaluated using one-way analysis of variance (ANOVA) with a Scheffe-type multiple comparison test. p values less than 0.05 were considered to be significant.

RESULTS AND DISCUSSION

CYP2C19 genetic differences had been reported in hydroxylation of omeprazole.[13)] In the present study, there was no information of *CYP2C19* genotype for each human liver microsome, however, comparative *in vitro* study on the metabolism of 3 PPIs could be conducted to validate further the difference among 3 PPIs in *CYP2C19* genotype-dependency on the phenotype. Two *in vitro* experimental approaches were performed; 1) the correlation of the rates of the 3 PPIs metabolism with CYP2C19 and CYP3A4 activities, 2) the inhibition of their metabolism by anti-CYP2C19 and anti-CYP3A4 antibodies.

There were good correlations between residual concentrations of both omeprazole and lansoprazole and the CYP2C19 activities in 5 lots of human liver microsomes (r=0.938 and r=0.962, respectively), whereas the residual concentration of rabeprazole was not (r=0.661) (Fig. 1). The hydroxylation of omeprazole and lansoprazole were also well correlated with CYP2C19 activities (r=914 and r=0.888, respectively) (Fig. 2). There was no correlation between the residual concentration of each PPI and the CYP3A4 activities (Fig. 3). Although it was demonstrated herein that CYP2C19 is responsible for the hydroxylation of both omeprazole and lansoprazole, these results were not adequate to explain that the *CYP2C19* genotype-dependent pharmacokinetics were more marked for omeprazole than lansoprazole.

Thus, the inhibition studies using anti-CYP2C19 and anti-CYP3A4 antibodies were additionally performed. As shown in Fig. 4, the hydroxylation of omeprazole by human liver microsomes was markedly inhibited by anti-CYP2C19 antibody in human liver microsomes (71.1—77.8%) and that of lansoprazole was less inhibited (54.4—68.6%). In contrast, their levels that omeprazole and lansoprazole were inhibited by anti-CYP3A4 antibody were similar. The sulfoxidations of omeprazole, lansoprazole and rabeprazole were escalated by anti-CYP2C19 antibody (103.9—136.7%, 115.5—135.1% and 105.1—112.1%, respectively), and significantly inhibited by anti-CYP3A4 antibody (0.0—18.6%, 0.0—48.8% and 49.0—60.5%, respectively) (Fig. 5).

The *in vitro* intrinsic clearance, maximum velocity (V_{max})/Michaelis constant (K_m), for stereoselective hydroxylation of (+)-omeprazole was reported to be 39.9 μl/mg/min, being extensively higher than its sulfoxidation (0.8 μl/mg/min), while other studies suggested that those for (+)-lansoplazole

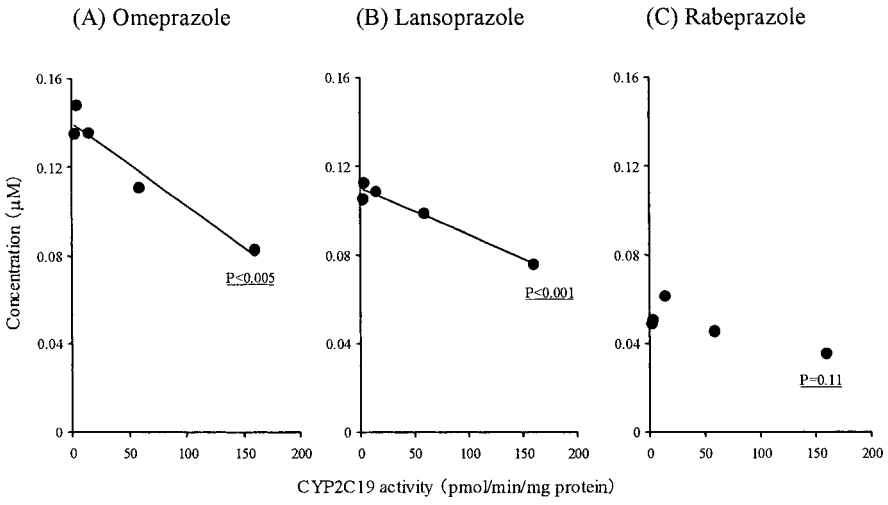

Fig. 1. Correlation between CYP2C19 Activity and the Residual Concentration of Omeprazole (A), Lansoprazole (B) and Rabeprazole (C) in 5 Lots of Human Liver Microsomes

Each 0.4 μM was incubated in the reaction buffer consisting of 10 mM of HEPES (pH 7.4), 2 mM of $MgCl_2$, 0.02 mM of EDTA 2Na and 0.22—0.26 mg/ml of either lot of microsomes for 30 min.

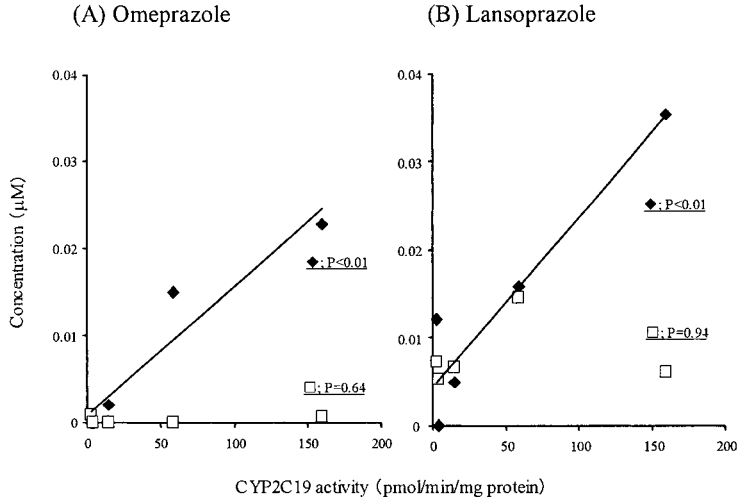

Fig. 2. Correlation between CYP2C19 Activity and Hydroxylation (◆) or Sulfoxidation (□) of Omeprazole (A) and Lansoprazole (B) in Human Liver Microsomes

Also see the legend to Fig. 1.

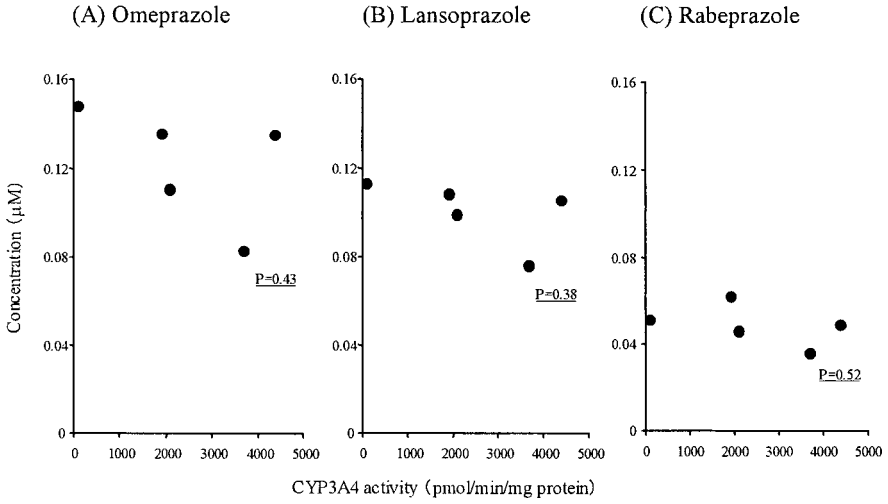

Fig. 3. Correlation between CYP3A4 Activity and the Residual Concentration of Omeprazole (A), Lansoprazole (B) and Rabeprazole (C) in Human Liver Microsomes

Also see the legend to Fig. 1.

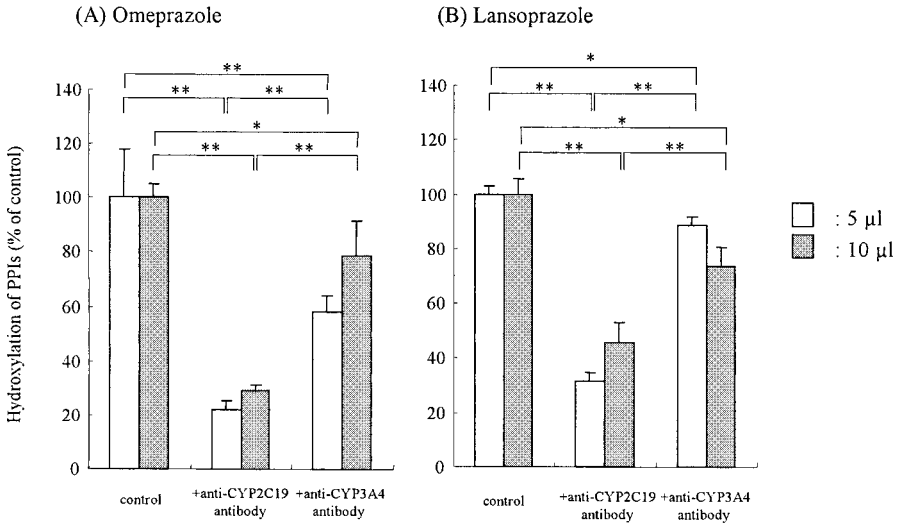

Fig. 4. Inhibition of Hydroxylation of Omeprazole (A) and Lansoprazole (B) by 5 or 10 μl of Anti-CYP2C19 and Anti-CYP3A4 Antibodies in Human Liver Microsomes

The values are the mean inhibition percentage (±S.D.) (n=3—4). The rates of metabolites formation in controls were 11.3—15.2 pmol/mg/min for omeprazole and 10.0—21.3 pmol/mg/min for lansoprazole. $*p<0.05$, $**p<0.01$: significantly different from the control group.

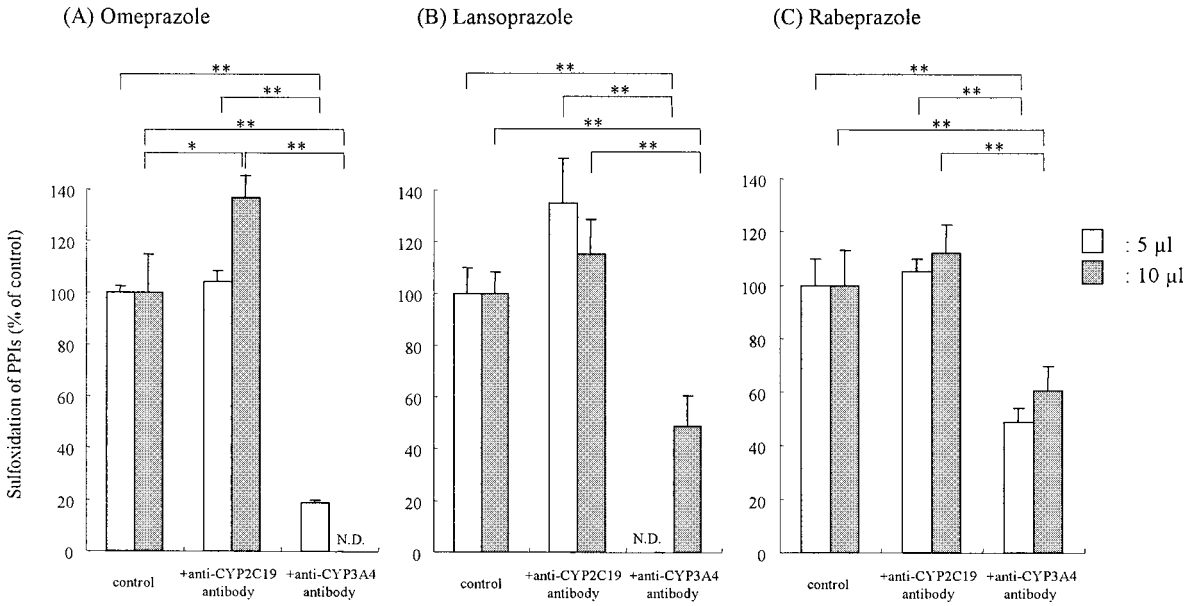

Fig. 5. Inhibition of Sulfoxidation of Omeprazole (A), Lansoprazole (B) and Rabeprazole (C) by 5 or 10 μl of Anti-CYP2C19 and Anti-CYP3A4 Antibodies in Human Liver Microsomes

The values are the mean inhibition percentage (±S.D.) (n=3—5). The rates of metabolites formation in controls were 5.2—5.8 pmol/mg/min for omeprazole and 1.6—11.9 pmol/mg/min for lansoprazole. $*p<0.05$, $**p<0.01$: significantly different from the control group. N.D.: not detected.

were 28.0 and 6.0 μl/mg/min, respectively.[14,15] Although, the experimental conditions among these studies were not identical, their findings were also consistent with those that showed the *CYP2C19* genotype-dependent pharmacokinetics was more marked for omeprazole than lansoprazole.

These results could explain the present clinical findings, especially for the comparative relationships between *CYP2C19* genotypes and the pharmacokinetics and pharmacodynamics among 3 PPIs. In conclusion, the present *in vitro* study could establish the relative contributions of CYP2C19 and CYP3A4 on total metabolism of PPIs and thereby help to explain that the *CYP2C19* genotype-dependent pharmacokinetics was more marked for omeprazole than lansoprazole.

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