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

Tomoko Kita, Toshiyuki Sakaeda, Takahiko Baba, Nobuo Aoyama, Mikio Kakumoto, Yoshie Kurimoto, Yuko Kawahara, Noboru Okamura, Shirou Kiriti, Masato Kasuga, and Katsuhiko Okumura

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. The subjects were classified into homo extensive metabolizers, hetero extensive metabolizers and poor metabolizers according to the CYP2C19 genotype. 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, 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. Cross reactivity of antibodies raised against CYP2C19 and CYP3A4 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 MgCl2, 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
(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 Millex-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 µg/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 MgCl2, 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 or 10 µl aliquot was injected into the HPLC system.

### RESULTS AND DISCUSSION

**CYP2C19** genetic differences had been reported in hydroxylation of omeprazole. 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 ($r_0=0.938$ and $r_0=0.962$, respectively), whereas the residual concentration of rabeprazole was not ($r_0=0.661$) (Fig. 1). The hydroxylation of omeprazole and lansoprazole were also well correlated with CYP2C19 activities ($r_0=0.914$ and $r_0=0.888$, 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 CYP2C19 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.

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

<table>
<thead>
<tr>
<th>P450 isozyme</th>
<th>Marker activity$^a$</th>
<th>Gender</th>
<th>Age</th>
<th>Lot# (HHM-)</th>
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<td></td>
<td></td>
<td>Female</td>
<td></td>
<td>0230</td>
</tr>
<tr>
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<td></td>
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<td></td>
<td>Pool</td>
<td></td>
<td>0264</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>Phacetin O-deethylation</td>
<td>291</td>
<td>51 years</td>
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<td>CYP2A6</td>
<td>Coumarin 7-hydroxylation</td>
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<td>29 years</td>
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<tr>
<td>CYP2C19</td>
<td>Mephenytoin 4’-hydroxylation</td>
<td>59</td>
<td>47 years</td>
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<tr>
<td>CYP2D6</td>
<td>Dextromethorphan O-demethylation</td>
<td>52</td>
<td>19 years</td>
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<td>CYP2E1</td>
<td>Chlorozoxone 6-hydroxylation</td>
<td>926</td>
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<td>CYP3A4</td>
<td>Testosteron 6β-hydroxylation</td>
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<td>CYP4A</td>
<td>Lauric acid 12-hydroxylation</td>
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</table>

$^a$ nmol/mg/min for CYP2A6, CYP3A4 and CYP4A; pmol/mg/min for others.
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 MgCl₂, 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.
were 28.0 and 6.0 μl/mg/min, respectively. 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.

REFERENCES