Effect of the CYP2D6*10 Genotype on Tolterodine Pharmacokinetics

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ABSTRACT:
This study was conducted to investigate the effect of the reduced function allele CYP2D6*10, which can be the cause of the intermediate metabolizer (IM), on tolterodine pharmacokinetics. Tolterodine is mainly metabolized to an active 5-hydroxymethyl metabolite (5-HM) by CYP2D6, and 5-HM is also metabolized by CYP2D6. Asian and white healthy volunteers (n = 108) received once daily multiple doses of tolterodine, and the serum concentrations of tolterodine and 5-HM were measured. All subjects were genotyped for CYP2D6. Tolterodine exposures [area under the curve (AUC)] increased in order of CYP2D6*1/*1 [extensive metabolizer (EM)] < CYP2D6*1/*10 < CYP2D6*10/*10 < CYP2D6*5/*10. It was expected that the order of 5-HM exposure would be reversed. However, the 5-HM AUC increased in the same order as that of tolterodine. This phenomenon was explained by considering CYP2D6 mediation of both production and elimination of 5-HM. The tolterodine and 5-HM exposures in CYP2D6*10/*10 were statistically higher than those for CYP2D6*1/*1 (3- and 1.5-fold, respectively). In CYP2D6*4/*4 [poor metabolizer (PM)], 5-HM was not produced and tolterodine exposure was 20-fold higher than that in CYP2D6*1/*1. With consideration for higher protein binding of tolterodine than 5-HM, the exposure as a sum of the unbound fraction of tolterodine and 5-HM (active moiety) in CYP2D6*10/*10 was 1.8-fold higher than that in CYP2D6*1/*1 and was also higher than that in CYP2D6*4/*4. Simulation using the values of EM and PM demonstrated that the maximum possible active moiety exposure was around the observed values of CYP2D6*5/*10, which were 1.9-fold higher than those for CYP2D6*1/*1. This is the first report to provide an example in which the IM shows higher exposure to pharmacological active moiety than the EM and PM.

Introduction
Tolterodine, (R)-N,N-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenylpropanamine, is an antimuscarinic drug for the treatment of overactive bladder. In humans, tolterodine is mainly eliminated by metabolism, with slight excretion into urine (Postlind et al., 1998). Two different oxidative metabolic pathways, hydroxylation and N-dealkylation have been identified in humans (Postlind et al., 1998). Hydroxylation to the pharmacologically active 5-hydroxymethyl metabolite (5-HM) is the predominant metabolic pathway, which is catalyzed by CYP2D6, whereas the minor N-dealkylation pathway is mediated by CYP3A (Postlind et al., 1998). 5-HM itself is metabolized by two major pathways of CYP2D6 and CYP3A4, and it is also renally excreted (Malhotra et al., 2008). Preclinical studies have demonstrated that 5-HM is pharmacologically equipotent with tolterodine in vitro and has similar functional bladder selectivity in vivo (Nilvebrant, 2000).

It is well established that CYP2D6 is subject to genetic polymorphism (http://www.cypalleles.ki.se/cyp2d6.htm). More than 70 CYP2D6 allelic variants have been discovered thus far (Zhou, 2009). They are classified as functional, nonfunctional, and reduced functional groups (Bradford, 2002). CYP2D6 allele frequency is known to vary among ethnic groups. In general, for Europeans, the functional group of alleles is predominant, with the presence of nonfunctional alleles mainly as CYP2D6*4. On the other hand, Asians and their close descendants have a low frequency of homozygous nonfunctional alleles, whereas they have a high frequency of the reduced function allele, CYP2D6*10 (Bradford, 2002; Ozawa et al., 2004; Ingelman-Sundberg, 2005; Bernard et al., 2006).

In extensive metabolizers (EMs) of CYP2D6, the mean systemic clearance of tolterodine was found to be 44 l/h with a half-life of 2 to 3 h (Brynne et al., 1998). In contrast, poor metabolizers (PMs) have 5 times lower clearance (9 l/h) with a mean half-life of 9 h. The levels of 5-HM are similar to those of tolterodine in EMs and are not quantifiable in PMs (Brynne et al., 1998). Despite differences in tolterodine pharmacokinetics between EMs and PMs, exposures to active moiety, the sum of unbound tolterodine and 5-HM, are comparable between EMs and PMs (Larsson et al., 1999) because of the 10-fold lower unbound fraction for tolterodine (3.7%) relative to that for 5-HM (36%) in serum (Påhlman and Gozzi, 1999). The clinical antimuscarinic effects of tolterodine are similar in CYP2D6 EMs and PMs (Van Kerrebroeck et al., 2001). Based on comparable exposure of active moiety and the clinical effects across EMs and PMs, the dose adjustment has not been necessary between EMs and PMs (Clemett and Jarvis, 2001).

ABBREVIATIONS: 5-HM, 5-hydroxymethyl metabolite of tolterodine; EM, extensive metabolizer; PM, poor metabolizer; IM, intermediate metabolizer; PR, prolonged release; AGP, α1-acid glycoprotein; AUC, area under the concentration-time curve; CI, confidence interval.
The effect of complete deficiency of CYP2D6 activity in PMs on the pharmacokinetics of tolterodine has been investigated (Brynne et al., 1998). However, there has been no investigation about the effect of reduced function alleles, such as CYP2D6*10, which can be the cause of an intermediate metabolizer (IM), on the pharmacokinetics of tolterodine and 5-HM. To our knowledge, this is the first report of a clinical study to demonstrate tolterodine and 5-HM exposures with the CYP2D6 polymorphism including the CYP2D6*10 allele.

In IMs of CYP2D6, it is expected that tolterodine exposure increases because of reduced enzyme activity. On the other hand, it is complicated to estimate the exposure of 5-HM. The active metabolite 5-HM is biotransformed from tolterodine by CYP2D6, and 5-HM itself is also metabolized by CYP2D6. When the activity of CYP2D6 is reduced, 5-HM exposure may decrease because of a decrease in the amount of 5-HM biotransformed from tolterodine, whereas 5-HM exposure may increase because of a reduction in clearance for 5-HM. Therefore, there is a possibility that IMs have the highest 5-HM exposure, and it can cause higher active moiety exposure in IMs than in EMs and PMs.

The aim of the present study was to investigate the effect of the CYP2D6 genotypes, especially the CYP2D6*10 allele, on the pharmacokinetics of tolterodine and 5-HM, with consideration of the opposite directional effects of reduced CYP2D6 enzyme activity, i.e., enhancement and reduction of 5-HM exposure. We also investigated the effect of the CYP2D6*10 allele on active moiety exposure among EMs, IMs, and PMs.

In addition, to investigate the magnitude of reduction in CYP2D6 intrinsic clearance in IMs, we estimated intrinsic clearances of CYP2D6 for tolterodine and 5-HM in each genotype from the data of the present study. Furthermore, to understand and describe the trend of change in exposures of 5-HM and active moiety by differences of CYP2D6 enzyme activity among various genotypes and to predict the maximum exposure, we simulated the exposures of tolterodine, 5-HM, and active moiety with changing CYP2D6 intrinsic clearance and compared the simulated results with the observed values.

Materials and Methods

Subjects. A total of 72 Asian (Japanese and Koreans, 24 male and 48 female) and 36 white (12 male and 24 female) subjects received tolterodine in this study. Concomitant use of any other medication was not permitted. The doses were administered as prolonged release (PR) capsules for 5 consecutive days.

CYP2D6 Genotyping. Genotyping was conducted via DNA purification from whole blood before the first dose, and DNA was extracted using the Gentra Puregene kit method. The presence of CYP2D6*3, CYP2D6*4, CYP2D6*5, and CYP2D6*10 was tested for each subject using multiplex polymerase chain reaction (CYP2D6*3 and *4), long-range polymerase chain reaction (CYP2D6*5), and allele-specific amplification (CYP2D6*10), respectively (PPGx, Cambridge, UK). If no variations were detected on an allele, it was defaulted to a CYP2D6*5 allele. The following genotypes were found in the subjects of the present study: CYP2D6*1/*1, CYP2D6*1/*10, CYP2D6*1/*4, CYP2D6*1/*5, CYP2D6*10/*10, CYP2D6*5/*10, and CYP2D6*4/*4. The expected phenotypes for each genotype were defined as follows: EM (CYP2D6*1/*1), IM (CYP2D6*1/*10, CYP2D6*1/*4, CYP2D6*1/*5, CYP2D6*10/*10, and CYP2D6*5/*10), and PM (CYP2D6*4/*4).

Pharmacokinetic Sampling. Venous blood samples for determination of tolterodine and 5-HM were drawn. Steady-state sampling was performed at 0, 0.5, 1, 2, 3, 4, 6, 9, 12, 18, and 24 h after the last administration. The blood samples (7 ml) were collected at each point in evacuated collection tubes without additives. The blood samples were allowed to coagulate for 45 min to 1 h, after which serum was separated by centrifugation (1000g for 10 min) at room temperature. The serum samples were stored frozen at −20°C until analyzed. A blood sample in connection with the predose sample on the last dosing day was taken for determination of α1c-acid glycoprotein (AGP).

Analytical Methods. Serum samples were assayed for tolterodine and 5-HM using a validated high-performance liquid chromatography with tandem mass spectrometry method (AABl Deutschland GmbH and Co. KG, Neu-Ulm, Germany). Stable isotope analogs of tolterodine [(R)-N,N-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-(pentadecoerophenyl) propanamine 1-tartrate] and 5-HM [N,N-diisopropyl-3-(2-hydroxy-5-hydroxymethylphenyl)-3-(pentadecyropheno) propanamine mandelate] were used as the internal standards, respectively. The compounds were separated from the serum by solid-phase extraction using octadecyl (C18) columns. The resulting extracts were analyzed by high-performance liquid chromatography with tandem mass spectrometry using a Zorbas SB-CN column mobile phase composed of 60% ammonium acetate (10 mM), 30% methanol, 10% acetonitrile, and 0.1% formic acid. The calibration standards for the serum concentration assay were linear over the range of 0.1 to 100 ng/ml. The lower limit of quantitation for both tolterodine and 5-HM was 0.1 ng/ml. Assay accuracy for tolterodine and 5-HM was 0.2 to 3.3% and 3.5 to 0.9%, respectively. Assay precision for tolterodine and 5-HM was 4.5 to 7.4 and 4.6 to 6.9%, respectively.

Pharmacokinetic Analysis. Pharmacokinetic parameters after multiple dosing were calculated based on serum concentrations of tolterodine and 5-HM. The calculations were performed by noncompartmental analysis using WinNonlin software (version 3.1; InnaPhase, Philadelphia, PA). The maximum serum concentration (Cmax) and area under the concentration-time curve from 0 to 24 h after the dose (AUC0–24) after multiple dosing were dose-normalized to 4 mg for the comparison among genotypes because the linear pharmacokinetics of tolterodine was confirmed up to 8 mg (Clemett and Jarvis, 2001).

Serum concentrations for active moiety were calculated using serum concentration data for tolterodine and 5-HM, and individual AGP concentrations. The fraction unbound (fu) was calculated as follows (Pahlman and Gozzi, 1999):

\[
\text{fu}_{\text{tolterodine}} = \frac{1}{1 + \left(2100 \times \text{AGP}(\text{g/l})/42\right)}
\]

\[
\text{fu}_{\text{5-HM}} = \frac{1}{1 + \left(130 \times \text{AGP}(\text{g/l})/42\right)}
\]

where fu_{tolterodine} and fu_{5-HM} are unbound fraction of tolterodine and 5-HM, respectively.

The concentration of active moiety = (fu_{tolterodine} × serum concentration of tolterodine) + (fu_{5-HM} × serum concentration of 5-HM). For calculation of active moiety concentration, serum concentrations of tolterodine and 5-HM were converted to nanomolar concentrations using the molecular weights of tolterodine (325.5) and 5-HM (341.5).

Estimation of Intrinsic Clearances of CYP2D6 for Tolterodine and 5-HM. The intrinsic clearances of CYP2D6 for tolterodine and 5-HM and the amount of tolterodine bioransformed to 5-HM were estimated by the following steps.

1. Estimation of intrinsic clearances of CYP2D6 for tolterodine and the amount of tolterodine bioransformed to 5-HM in each CYP2D6 genotype.

Intrinsic clearance of tolterodine (CL_{int,tolterodine}) in each CYP2D6 genotype, CYP2D6*1/*1, CYP2D6*1/*10, CYP2D6*1/*4 or CYP2D6*1/*5, CYP2D6*10/*10, CYP2D6*5/*10, and CYP2D6*4/*4, was estimated from the individual dose-normalized tolterodine AUC_{0–24} and individual fu_{tolterodine} in the present study. The estimation was based on a parallel tube model with assumption of ignorable renal clearance because of low urinary excretion (<3%) (Brynne et al., 1998) and fraction absorbed (fa) × intestinal availability (fg) equaled 1. The fa × fg for tolterodine was assumed to be almost 1 because it was reported that bioavailability of tolterodine in PMs was 0.91 (Brynne et al., 1998; Van Kerrebroeck et al., 2001). The parallel tube model was used for the estimation because the mean of fu_{tolterodine} × intrinsic clearance of CYP2D6 for tolterodine (CL_{int,CYP2D6,tolterodine}) in CYP2D6*1/*1 was estimated to be 157 l/h, which was higher than the hepatic blood flow rate [Qh = 91.8 l/h (Kato et al., 2003)] (To and Houston, 2004). The median CL_{int,tolterodine} in two subjects of CYP2D6*4/*4 genotype in the present study was used as the CL_{int} of tolterodine by a metabolic
pathway other than CYP2D6 (CLint_{other, tolterodine}). It was confirmed that the median CLint_{other, tolterodine} in CYP2D6*4/*4 in the present study (436 l/h) was consistent with the estimation (443 l/h) from the total clearance in PMs after intravenous infusion of tolterodine (Bryne et al., 1998). CLint_{CYP2D6, tolterodine} in each genotype was calculated by subtracting CLint_{other, tolterodine} from estimated individual CLint_{tolterodine}. The amount of 5-HM biotransformed from tolterodine was calculated by using the estimated individual CLint_{CYP2D6, tolterodine}. Dosage of tolterodine was set at 2.74 mg as tolterodine, which corresponded to 4 mg of tolterodine tartrate. The dose-normalized Cmax and AUC0–24 of tolterodine, 5-HM, and active moiety after multiple dosing are summarized in Table 2 and are plotted in Figs. 1, 2, and 3, respectively. The mean values of dose-normalized Cmax and AUC0–24 of tolterodine in CYP2D6*1/*10 were similar to those in CYP2D6*1/*1, whereas those of CYP2D6*1/*4 or CYP2D6*1/*5 were approximately 1.9- and 2-fold higher than that of CYP2D6*1/*1 (Table 2). Both Cmax and AUC0–24 in CYP2D6*10/*10 were significantly higher than those in CYP2D6*1/*1 and CYP2D6*1/*10 (Fig. 1). They were approximately 2.6- and 3-fold higher than those in CYP2D6*1/*1, respectively. The individual Cmax and AUC0–24 values for two subjects of CYP2D6*5/*10 were higher than the mean values for those of CYP2D6*10/*10. The individual Cmax and AUC0–24 values of CYP2D6*4/*4 were approximately 10- and 20-fold higher than the mean values of CYP2D6*1/*1, respectively. The values of CYP2D6*5/*10 and

Statistical Analysis. Statistical analysis for dose-normalized Cmax and AUC0–24 of tolterodine, 5-HM, and active moiety among genotypes was performed by one-way analysis of variance and a Bonferroni post hoc test with SAS (version 8.02; SAS Institute Inc., Cary, NC). CYP2D6*5/*10 and CYP2D6*4/*4 were not included in the analysis of variance because only two subjects were classified as each genotype. Differences were considered to be statistically significant when P < 0.05. In addition, simulation of the change of exposures was conducted using SAS.

Genotyping. The allele frequencies of CYP2D6*10 in Asian and white subjects were 40 and 1.4%, respectively. The allele frequency of CYP2D6*5 was 5.6% in Asians and 1.4% in whites. There were no Asian subjects who had the CYP2D6*4 allele, but the allele frequency of CYP2D6*4 in whites was 16.7%. Demographics of subjects in each genotype are provided in Table 1.

Pharmacokinetics. The dose-normalized Cmax and AUC0–24 of tolterodine, 5-HM, and active moiety after multiple dosing are summarized in Table 2 and are plotted in Figs. 1, 2, and 3, respectively. The mean values of dose-normalized Cmax and AUC0–24 of tolterodine in CYP2D6*1/*10 were similar to those in CYP2D6*1/*1, whereas those of CYP2D6*1/*4 or CYP2D6*1/*5 were approximately 1.9- and 2-fold higher than that of CYP2D6*1/*1 (Table 2). Both Cmax and AUC0–24 in CYP2D6*10/*10 were significantly higher than those in CYP2D6*1/*1 and CYP2D6*1/*10 (Fig. 1). They were approximately 2.6- and 3-fold higher than those in CYP2D6*1/*1, respectively. The Cmax and AUC0–24 values for two subjects of CYP2D6*5/*10 were higher than the mean values for those of CYP2D6*10/*10. The individual Cmax and AUC0–24 values of CYP2D6*4/*4 were approximately 10- and 20-fold higher than the mean values of CYP2D6*1/*1, respectively. The values of CYP2D6*5/*10 and

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**TABLE 1**

Demographics of subjects of each genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Male Gender</th>
<th>Age</th>
<th>Height</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1/*1</td>
<td>45</td>
<td>20 (44.4%)</td>
<td>32.9 (10.1)</td>
<td>166.7 (8.9)</td>
<td>62.1 (8.1)</td>
</tr>
<tr>
<td>*1/*10</td>
<td>31</td>
<td>6 (19.4%)</td>
<td>33.3 (9.6)</td>
<td>162.8 (8.7)</td>
<td>58.0 (7.6)</td>
</tr>
<tr>
<td>*1/*4 or *1/*5</td>
<td>15</td>
<td>3 (20.6%)</td>
<td>36.3 (10.2)</td>
<td>162.8 (8.0)</td>
<td>56.1 (5.7)</td>
</tr>
<tr>
<td>*1/*10</td>
<td>13</td>
<td>5 (38.5%)</td>
<td>33.7 (10.1)</td>
<td>165.0 (7.7)</td>
<td>56.4 (6.9)</td>
</tr>
<tr>
<td>*5/*10</td>
<td>2</td>
<td>1 (50.0%)</td>
<td>20, 43</td>
<td>150, 180</td>
<td>58, 64</td>
</tr>
<tr>
<td>*4/*4</td>
<td>2</td>
<td>1 (50.0%)</td>
<td>21, 25</td>
<td>160, 161</td>
<td>57, 66</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD) except for gender. If the number of subject is less than 2, individual values are shown instead of a descriptive summary.

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**TABLE 2**

Dose-normalized Cmax and AUC of tolterodine, 5-HM, and active moiety in each genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cmax</th>
<th>AUC0–24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolerodine</td>
<td>5-HM</td>
<td>Active Moiety</td>
</tr>
<tr>
<td>*1/*1 (n = 45)</td>
<td>1.65 (2.66)</td>
<td>0.85–2.45</td>
</tr>
<tr>
<td>*1/*10 (n = 31)</td>
<td>1.64 (0.92)</td>
<td>1.30–1.98</td>
</tr>
<tr>
<td>*1/*4 or *1/*5 (n = 15)</td>
<td>3.18 (2.08)</td>
<td>20.3–4.33</td>
</tr>
<tr>
<td>*1/*10 (n = 13)</td>
<td>4.31 (2.87)</td>
<td>2.58–6.04</td>
</tr>
<tr>
<td>*5/*10 (n = 2)</td>
<td>6.06, 6.34</td>
<td>79.04, 87.09</td>
</tr>
<tr>
<td>*4/*4 (n = 2)</td>
<td>17.82, 18.84</td>
<td>346.10, 358.94</td>
</tr>
</tbody>
</table>

N.A., not applicable.

* Significantly higher than CYP2D6*1/*1 using the Bonferroni post hoc test (P < 0.05).
† Significantly higher than CYP2D6*1/*10.
CYP2D6*4/*4 were not included in the 95% confidence interval (CI) of CYP2D6*10/*10 (Table 2).

The metabolite 5-HM was unquantifiable in the subjects of CYP2D6*4/*4. In other genotypes, C<sub>max</sub> values were distributed within the same range (Fig. 2). The mean values of AUC<sub>0–24</sub> tended to increase in the following order: CYP2D6*1/*1, CYP2D6*1/*10, CYP2D6*1/*4 or CYP2D6*1/*5, CYP2D6*10/*10, and CYP2D6*5/*10 (Table 2). The mean value of CYP2D6*1/*4 was similar to that of CYP2D6*1/*1. The mean AUC<sub>0–24</sub> values of CYP2D6*1/*4 or CYP2D6*1/*5 and CYP2D6*10/*10 were 1.2- and 1.5-fold higher than the value in CYP2D6*1/*1, respectively. The difference between CYP2D6*1/*1 and CYP2D6*10/*10 was statistically significant. The individual values of CYP2D6*5/*10 were similar to the mean of CYP2D6*10/*10, but they were not included in the 95% CI of CYP2D6*1/*1 (Table 2).

As active moiety, the mean values of C<sub>max</sub> and AUC<sub>0–24</sub> tended to increase in the following order: CYP2D6*1/*1, CYP2D6*1/*10, CYP2D6*1/*4 or CYP2D6*1/*5, CYP2D6*10/*10, and CYP2D6*5/*10 (Table 2). The mean C<sub>max</sub> value of CYP2D6*10/*10 was 1.4-fold higher than that of CYP2D6*1/*1, and it was statistically significant (Fig. 3). The mean AUC<sub>0–24</sub> values of CYP2D6*1/*10 and CYP2D6*1/*4 or CYP2D6*1/*5 were 1.3- and 1.5-fold higher than the value of CYP2D6*1/*1, respectively, and these differences were statistically significant. The AUC<sub>0–24</sub> of CYP2D6*1/*10 was 1.8-fold higher than that of CYP2D6*1/*1 and was significantly higher than those of CYP2D6*1/*1 and CYP2D6*1/*10. The individual values of CYP2D6*5/*10 were included in the 95% CI of CYP2D6*10/*10 but were higher than that of CYP2D6*1/*1. The individual exposures in CYP2D6*4/*4 were distributed between the mean of CYP2D6*1/*1 and CYP2D6*1/*10, and they were not included in the 95% CI of CYP2D6*1/*1.

**Estimation of Intrinsic Clearances of CYP2D6 for Tolterodine and 5-HM Metabolism.** CL<sub>int</sub> for tolterodine and 5-HM metabolism in each genotype can be estimated from the AUC values using the liver model. The estimated CL<sub>int</sub> for tolterodine and 5-HM metabolism in each genotype are shown in Table 3. CL<sub>int</sub> for tolterodine and 5-HM in IMs (CYP2D6*1/*10, CYP2D6*1/*4 or CYP2D6*1/*5, CYP2D6*10/*10, and CYP2D6*5/*10) were higher than that of CYP2D6*1/*1, and it was statistically significant (Fig. 3). The mean AUC<sub>0–24</sub> values of CYP2D6*1/*10 and CYP2D6*1/*4 or CYP2D6*1/*5 were 1.3- and 1.5-fold higher than the value of CYP2D6*1/*1, respectively, and these differences were statistically significant. The AUC<sub>0–24</sub> of CYP2D6*1/*10 was 1.8-fold higher than that of CYP2D6*1/*1 and was significantly higher than those of CYP2D6*1/*1 and CYP2D6*1/*10. The individual values of CYP2D6*5/*10 were included in the 95% CI of CYP2D6*10/*10 but were higher than that of CYP2D6*1/*1. The individual exposures in CYP2D6*4/*4 were distributed between the mean of CYP2D6*1/*1 and CYP2D6*1/*10, and they were not included in the 95% CI of CYP2D6*1/*1.

**FIG. 1.** Dose-normalized C<sub>max</sub> and AUC of tolterodine in each genotype. Left panel shows C<sub>max</sub> and right panel shows AUC<sub>0–24</sub>. The box edges show the 25th and 75th percentiles. The whiskers extend from each box edge to the furthest data within a distance of 1.5 interquartile ranges from the 25th and 75th percentiles. The open circles outside the whiskers represent outliers. *, significantly higher than CYP2D6*1/*1 (P < 0.05), using the Bonferroni post hoc test. †, significantly higher than CYP2D6*1/*10 (P < 0.05).

**FIG. 2.** Dose-normalized C<sub>max</sub> and AUC of 5-HM in each genotype. Left panel shows C<sub>max</sub> and right panel shows AUC<sub>0–24</sub>. The box edges show the 25th and 75th percentiles. The whiskers extend from each box edge to the furthest data within a distance of 1.5 interquartile ranges from the 25th and 75th percentiles. The open circles outside the whiskers represent outliers. *, significantly higher than CYP2D6*1/*1 (P < 0.05), using the Bonferroni post hoc test. †, significantly higher than CYP2D6*1/*10 (P < 0.05).
CYP2D*5/*10) were estimated to be lower than those in EMs (CYP2D6*1/*1). For both tolterodine and 5-HM, the relative enzyme activities compared with CYP2D6*1/*1 in CYP2D6*1/*10, CYP2D6*1/*4 or CYP2D6*1/*5, and CYP2D6*10/*10 were estimated to be approximately 70, 50, and 40%, respectively, of that of CYP2D6*1/*1 (Table 3). Individual relative activity in two subjects of CYP2D6*5/*10 were less than 30%.

Simulation of the Exposures of Unbound Tolterodine, 5-HM, and Active Moiety with Changing Intrinsic Clearance of CYP2D6. Once CLint values for EMs (CYP2D6*1/*1) and PMs (CYP2D6*4/*4) are determined, the AUC in IMs can be simulated by changing CLintCYP2D6. Shown in Fig. 4. The observed mean AUCs of each tolterodine, unbound 5-HM, and active moiety with changing CLintCYP2D6 and fell to zero. As active moiety (the sum of the fu5-HM, CYP2D6*1/*4 or CYP2D6*1/*5) were estimated to be approximately 70, 50, and 40%, respectively, of that of CYP2D6*1/*1 (Table 3). Individual relative activity in two subjects of CYP2D6*5/*10 were less than 30%.

In the simulation, tolterodine AUC continued to increase from the exposure in EMs (CYP2D6*1/*1) to the exposure in PMs (CYP2D6*4/*4) with decreasing CLintCYP2D6. For 5-HM, the level in EMs, AUC increased to the peak with decreasing enzyme activity at approximately 80% reduction in CLintCYP2D6 and fell to zero. As active moiety (the sum of the unbound fraction of tolterodine and 5-HM), the exposure described a curve with the peak at approximately 90% reduction in CLintCYP2D6, and the exposure at the peak was 1.9-fold higher than that in EMs. Simulation showed that the exposures in EMs and PMs were almost comparable, whereas those in IMs (CYP2D6*1/*10, CYP2D6*1/*4 or CYP2D6*1/*5, CYP2D6*10/*10, and CYP2D6*5/*10) who had reduced enzyme activity were higher than the exposures in EMs and PMs. The simulated AUCs in IMs were well matched with the observed IM values in the present study and the highest simulated active moiety exposure was around the observed value of CYP2D6*5/*10.

We used the reported mean values of fuFutolterodine and fu5-HM for this simulation (see Materials and Methods). When fuFutolterodine and fu5-HM are changed from minimum (fuFutolterodine = 0.016 and fu5-HM = 0.21) to maximum (0.063 and 0.52, respectively) in the present study, the maximum increase in active moiety from EMs (2.3-fold) was similar to the result for mean fuFutolterodine and fu5-HM (1.9-fold).

**Discussion**

This is the first report of a clinical study to demonstrate the impact of the CYP2D6*10 allele on tolterodine and 5-HM exposures. We also provided the first example for which IMs shows higher exposure to pharmacological active moiety than EMs and PMs.

In the present study, we found spontaneous incidences of CYP2D6*10 in Asians (40%) similar to previous reports (Bradford, 2002; Ozawa et al., 2004; Ingelman-Sundberg, 2005; Bernard et al., 2006). Some deficient or reduced mutations of CYP2D6 other than CYP2D6*10, such as CYP2D6*14, CYP2D6*21, and CYP2D6*41, had been reported in Asian populations (Yamazaki et al., 2003; Kubo et al., 2007; Sakuyama et al., 2008). In our study, one Asian subject classified as CYP2D6*1/*4 showed higher serum concentrations of tolterodine and lower concentrations of 5-HM. There is a possibility that this subject had one of these mutations. The existence of such reduced unidentified mutations in CYP2D6*1/*1 might make it more difficult to detect the differences in exposures between CYP2D6*1/*1 and IMs (CYP2D6*1/*10, CYP2D6*1/*4 or CYP2D6*1/*5, CYP2D6*10/*10, and CYP2D6*5/*10). Nevertheless, in the present study, a significant difference between CYP2D6*1/*1 and CYP2D6*10/*10 was observed.

The tolterodine and 5-HM pharmacokinetic parameters of CYP2D6*1/*1 and the homozygous genotype of the nonfunctional allele (CYP2D6*4/*4) were comparable with those for EMs and PMs.

**Table 3**

Estimated intrinsic clearances of CYP2D6 for tolterodine and for 5-HM and the relative enzyme activity to CYP2D6*1/*1 in each genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Tolterodine CLintCYP2D6, tolterodine (l/h)</th>
<th>Relative Enzyme Activity</th>
<th>5-HM CLintCYP2D6, 5-HM (l/h)</th>
<th>Relative Enzyme Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1/*1 (n = 45)</td>
<td>4441 (1479)</td>
<td>100</td>
<td>131 (55)</td>
<td>100</td>
</tr>
<tr>
<td>*1/*10 (n = 31)</td>
<td>2989 (1136)</td>
<td>65</td>
<td>89 (59)</td>
<td>68</td>
</tr>
<tr>
<td>*1/*4 or *1/*5 (n = 15)</td>
<td>2121 (984)</td>
<td>48</td>
<td>68 (43)</td>
<td>52</td>
</tr>
<tr>
<td>*1/*10 (n = 13)</td>
<td>1594 (1287)</td>
<td>36</td>
<td>48 (51)</td>
<td>37</td>
</tr>
<tr>
<td>*5/*10 (n = 2)</td>
<td>435, 729</td>
<td>10, 16</td>
<td>0, 33</td>
<td>0, 25</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD). If the number of subject is less than 2, individual values are shown instead of a descriptive summary.
Tolterodine moiety exposures were almost the same between reported previously (Olsson and Szamosi, 2001). As a result, active rate of CL\text{int}\text{CYP2D6} from 5-HM (blue), and active moiety (red) after tolterodine administration and reduction /H\text{18528} CYP2D6*4/*4 respectively. Mean values of observed AUC of unbound tolterodine, unbound 5-HM, and active moiety are shown by the green, blue, and red solid line, F(100%, relative activity in Table 3) as closed symbols with S.D. Individual values of CYP2D6*4/*4 were the same.

With regard to the magnitude of enzyme activity reduction, the relative enzyme activity to CYP2D6*1/*1 in each genotype was estimated from the data in the present study. Activities of CYP2D6*1/1\textit{10}, CYP2D6*1/*4 or CYP2D6*1/*5, and CYP2D6*10/*10 were approximately 70, 50, and 40%, respectively, of that of CYP2D6*1/*1, both for tolterodine and 5-HM. Although Shen et al. (2007) reported that the extent of decrease in enzyme activity of CYP2D6*10 in comparison with that of CYP2D6*1 was different among various substrates in vitro, at least for tolterodine and 5-HM, it was expected that the relative enzyme activities of CYP2D6*10 and CYP2D6*1 were the same.

In the simulation with the estimated CL\text{int}\text{CYP2D6}, in contrast with a continuous increase in tolterodine exposure with decreasing enzyme activity, the 5-HM exposure showed a curve with a peak (Fig. 4). It was considered that the effect of enzyme activity reduction on elimination of 5-HM (enhancement of the exposure) was dominant in the area from mild to moderate reduction and that the reducing effect on production of 5-HM (reduction of the exposure) became dominant when the reduction was excessive. From our simulation results, the peak of 5-HM exposure after administration of tolterodine was estimated to be approximately 80% reduction in CL\text{int}\text{CYP2D6}; it was between that of CYP2D6*10/*10 and CYP2D6*5/*10 in the present study. When the enzyme activity reduction was mild to moderate, active moiety exposure increased with decreasing enzyme activity because both tolterodine and 5-HM increased with decreasing enzyme activity. When the enzyme activity reduction was excessive, 5-HM exposure fell to zero, but active moiety exposure was still higher than

5-HM exposure would be reversed. However, the 5-HM AUC increased in the same order as the tolterodine AUC except for CYP2D6*4/*4.

The active metabolite 5-HM is the metabolite of tolterodine produced by CYP2D6, and 5-HM itself is also metabolized by CYP2D6. When the activity of CYP2D6 decreases, 5-HM exposure may decrease because of the decreased amount of 5-HM biotransformed from tolterodine (reduction of the exposure), whereas 5-HM exposure may increase because of the reduction in clearance of 5-HM (enhancement of the exposure) (Fig. 5). 5-HM exposure in subjects with a reduced function allele depends on the balance of opposite directional effects, reduction and enhancement of 5-HM exposure.

As expected from enzyme activity reduced by CYP2D6*10 in in vitro studies (Yokota et al., 1993; Johansson et al., 1994), the tolterodine exposure increased in the order of CYP2D6*1/*1 < CYP2D6*1/*10 < CYP2D6*1/*4 or CYP2D6*1/*5 < CYP2D6*10/*10 < CYP2D6*5/*10 < CYP2D6*4/*4. It was expected that the order of tolterodine exposure increased in the order of CYP2D6*1/*1 < CYP2D6*1/*10 < CYP2D6*1/*4 or CYP2D6*1/*5 < CYP2D6*10/*10 < CYP2D6*5/*10 < CYP2D6*4/*4, as reported previously.

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As expected from enzyme activity reduced by CYP2D6*10 in in vitro studies (Yokota et al., 1993; Johansson et al., 1994), the tolterodine exposure increased in the order of CYP2D6*1/*1 < CYP2D6*1/*10 < CYP2D6*1/*4 or CYP2D6*1/*5 < CYP2D6*10/*10 < CYP2D6*5/*10 < CYP2D6*4/*4, as reported previously.
that in CYP2D6*1/*1, compensated for by the steep increase in tolterodine. As a result, it was simulated that all IMs (CYP2D6*1/*10, CYP2D6*1/*4 or CYP2D6*1/*5, CYP2D6*10/*10, and CYP2D6*5/*10) had higher active moiety exposure than that in EMs (CYP2D6*1/*1) and PMs (CYP2D6*4/*4). This simulation result was well matched with observed phenomena in the present study and the highest simulated active moiety exposure was around the observed value of CYP2D6*5/*10.

With regard to the C_{max} of 5-HM, no differences were observed among genotypes although AUCs in IMs were higher than those in EMs. A possible explanation is as follows. The formulation was a sustained-release formulation and the release of tolterodine could be considered as an infusion. The 5-HM C_{max} is determined by this infusion rate and clearances for tolterodine and 5-HM, which are influenced by genotype. On the other hand, the 5-HM AUC is determined by only the clearances. Under this circumstance, it is possible that the C_{max} values observed were the same, but AUCs were different among genotypes.

In the present study, as a result of a 3-fold increase in tolterodine exposure and a 1.5-fold increase in 5-HM exposure, active moiety exposure, which is the sum of the unbound fraction of tolterodine and 5-HM, which corresponded to 4 mg of tolterodine tartrate (molecular weights for tolterodine and tolterodine tartrate are 325.5 and 475.6).

The amount of 5-HM biotransformed from tolterodine (Dose_{5-HM}) was calculated as follows:

\[
Dose_{5-HM} = Dose \times \frac{fa_{tolterodine} \times MW_{tolterodine}}{MW_{5-HM}}
\]

where Qh is hepatic blood flow rate, fu is fraction unbound, CLint is intrinsic clearance of each enzyme, fa is fraction absorbed, and fg is intestinal availability. The subscript “other” indicates a hepatic pathway other than CYP2D6, which corresponds to the hepatic pathway in PMs. The following values were used for these estimations: Qh was calculated as 91.8 l/h from 25 ml/min/kg (Kato et al., 2003) and 60 kg b.wt., as approximately equal to the mean body weight of the present study. The fu of tolterodine (fu_{tolterodine}) was calculated from individual AGP concentrations as described under Materials and Methods. Clearances were adjusted by the blood/serum concentration ratio (RB_{tolterodine} = 0.6). The median value of CLint_{tolterodine} of CYP2D6*4/*4 in the present study, 436 l/h, was used as CLint_{other,tolterodine}.

The dose was set at 2.74 mg as tolterodine, which corresponded to 4 mg of tolterodine tartrate (molecular weights for tolterodine and tolterodine tartrate are 325.5 for tolterodine and 475.5 for 5-HM).

### Appendix 2

Equations for the estimation of intrinsic clearance of CYP2D6 for 5-HM in each CYP2D6 genotype (parallel tube model) are as follows:

\[
CL_{int5-HM} = CL_{inttolterodine} \times \frac{f_{5-HM}}{fu_{5-HM}}
\]

where \(f_{5-HM}\) is the ratio of the contribution of CYP2D6 to the P450-mediated tolterodine clearance and MW is molecular weight (325.5 for tolterodine and 475.5 for 5-HM).

### Appendix 1

Equations for the estimation of intrinsic clearance of CYP2D6 for tolterodine and the amount of tolterodine biotransformed to 5-HM in each CYP2D6 genotype (parallel tube model) are as follows:

\[
CL_{inttolterodine} = \frac{CL_{inttolterodine} \times MW_{tolterodine}}{MW_{5-HM}}
\]

\[
CL_{int5-HM} = CL_{inttolterodine} - CL_{intother,tolterodine}
\]

where Qh is hepatic blood flow rate, fu is fraction unbound, CLint is intrinsic clearance of each enzyme, fa is fraction absorbed, and fg is intestinal availability. The subscript “other” indicates a hepatic pathway other than CYP2D6, which corresponds to the hepatic pathway in PMs.

The following values were used for these estimations: Qh was calculated as 91.8 l/h from 25 ml/min/kg (Kato et al., 2003) and 60 kg b.wt., as approximately equal to the mean body weight of the present study. The fu of tolterodine (fu_{tolterodine}) was calculated from individual AGP concentrations as described under Materials and Methods. Clearances were adjusted by the blood/serum concentration ratio (RB_{tolterodine} = 0.6). The median value of CLint_{tolterodine} of CYP2D6*4/*4 in the present study, 436 l/h, was used as CLint_{other,tolterodine}.

The dose was set at 2.74 mg as tolterodine, which corresponded to 4 mg of tolterodine tartrate (molecular weights for tolterodine and tolterodine tartrate are 325.5 for tolterodine and 475.5 for 5-HM).

### Appendix 3

Simulation of the exposures of unbound tolterodine, 5-HM, and active moiety with changing intrinsic clearance of CYP2D6 is as follows:
where \( \text{C}_{\text{int}} \text{CYP2D6, tolterodine} \) and \( \text{C}_{\text{int}} \text{CYP2D6, 5-HM} \) were changed from no reduction, which was the mean value of \( \text{CYP2D6}^*1/1^* \) in the present study (4441 l/h for tolterodine and 130.6 l/h for 5-HM, respectively) to 100% reduction equal to the condition in PMs. For all other parameters, the same values as in Appendices 1 and 2 were used.

References


Ingelman-Sundberg M (2005) Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. Pharmacogenomics J 5:6–13.


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