Effect of the \textit{CYP2D6*10} Genotype on Tolterodine Pharmacokinetics.

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List of nonstandard abbreviations:
5-HM, 5-hydroxymethyl metabolite of tolterodine
Abstract
This study was conducted to investigate the effect of the reduced function allele \textit{CYP2D6*10} which can cause intermediate metabolizer (IM) on tolterodine pharmacokinetics. Tolterodine is mainly metabolized to active 5-hydroxymethyl metabolite (5-HM) by CYP2D6 and 5-HM is also metabolized by CYP2D6. Asian and Caucasian 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 (AUC) increased in order of \textit{CYP2D6*1/*1} (extensive metabolizer, EM) \textless \textit{CYP2D6*1/*10} \textless \textit{CYP2D6*10/*10} \textless \textit{CYP2D6*5/*10}. It was expected that the order of 5-HM exposure would be reversed. However, 5-HM AUC increased in the same order as tolterodine. This phenomenon was explained by considering the CYP2D6 mediation of both production and elimination of 5-HM. The exposures of tolterodine and 5-HM in \textit{CYP2D6*10/*10} were statistically higher than \textit{CYP2D6*1/*1} (3- and 1.5-fold, respectively). In \textit{CYP2D6*4/*4} (poor metabolizer, PM), 5-HM was not produced and exposure of tolterodine was 20-fold higher than \textit{CYP2D6*1/*1}. With consideration for higher protein binding of tolterodine than 5-HM, the exposure as sum of unbound fraction of tolterodine and 5-HM (active moiety) in \textit{CYP2D6*10/*10} was 1.8-fold higher than \textit{CYP2D6*1/*1} and was also higher than \textit{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 \textit{CYP2D6*5/*10} which was 1.9-fold higher than \textit{CYP2D6*1/*1}. This is the first report to provide the example of which IM shows higher exposure to pharmacological active moiety than 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). There are two different oxidative metabolic pathways, hydroxylation and \(N\)-dealkylation, that 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 catalysed by cytochrome P450 (CYP) 2D6, while 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 compared 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). Over 70 CYP2D6 allelic variants have been discovered thus far (Zhou, 2009). They are classified as functional, non-functional and reduced functional groups (Bradford, 2002). CYP2D6 allele frequency is known to vary among ethnic groups. Generally, for Europeans, the functional group of alleles are predominant with the presence of non-functional alleles mainly as CYP2D6*4. On the other hand, Asians and their close descendants have a low frequency of homozygous of non-functional 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 (EM) of CYP2D6, the mean systemic clearance of tolterodine was found to be 44 L/h with a half-life of 2–3 h (Brynne et al., 1998). In contrast, poor metabolizers (PM) 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 EM and not quantifiable in PM.
(Brynne et al., 1998). Despite differences in tolterodine pharmacokinetics between EM and PM, exposures to active moiety, the sum of unbound tolterodine and 5-HM, are comparable between EM and PM (Larsson et al., 1999) due to the 10-fold lower unbound fraction for tolterodine (3.7%) relative to 5-HM (36%) in serum (Pahlman and Gozzi, 1999). The clinical antimuscarinic effects of tolterodine are similar in CYP2D6 EMs and PMs (Van Kerrebroeck et al., 2001). Based on the 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).

The effect of complete deficiency of CYP2D6 activity in PM 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 intermediate metabolizer (IM), on the pharmacokinetics of tolterodine and 5-HM. To our knowledge, this is the first report of clinical study to demonstrate tolterodine and 5-HM exposures with CYP2D6 polymorphism including CYP2D6 *10 allele.

In IM of CYP2D6, it is expected that tolterodine exposure increases due to 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 reduces, 5-HM exposure may decrease due to decreasing the amount of 5-HM biotransformed from tolterodine, whereas exposure of 5-HM may increase due to reduction of clearance for 5-HM. Therefore, there is a possibility that IM has the highest exposure of 5-HM and it can cause higher active moiety exposure in IM than EM and PM.

The aim of the present study was to investigate the effect of the CYP2D6 genotypes, especially for 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 CYP2D6 *10 allele on active moiety exposure, the sum of unbound tolterodine and 5-HM, 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 Caucasian (12 male and 24 female) subjects received tolterodine administration in this study. Concomitant use of any other medication was not permitted. The study was performed in accordance with the latest revisions to the Declaration of Helsinki, ethical approval having been obtained from the local Institutional Review Board of Health and Regulatory Affairs Authority. All volunteers gave their written informed consent prior to commencement of the study.

Study design

This was a randomized, multiple-dose study in healthy Asian and Caucasian subjects. The doses were administered as prolonged release capsules (PR). There were three dose groups of 2-, 4-, and 6-mg tolterodine L-tartrate, each group including 36 subjects. Each subject received daily single doses of tolterodine PR for 5 consecutive days.

CYP2D6 genotyping

Genotyping was conducted via DNA purification from whole blood prior to the first dosing and DNA was extracted using Gentra ‘PureGene’ kit method. The presence of CYP2D6*3, CYP2D6*4, CYP2D6*5 and CYP2D6*10 was tested for each subject. They were determined 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 wild type (CYP2D6*1) assignment. 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 carried out at 0, 0.5, 1, 2, 3, 4, 6, 9, 12, 18 and 24 hours 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 minutes to 1 hour, after which serum was separated by centrifugation (1,000 x g for 10 minutes) at room temperature. The serum samples were stored frozen at –20°C until analyzed.

A blood sample in connection with the pre-dose sample on the last dosing day was taken for determination of α₁-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 (LC/MS/MS) method (AAI Deutschland GmbH & Co. KG, Germany). Stable isotope analogues of tolterodine ((R)- N,N- diisopropyl- 3-(2-hydroxy-5-methylphenyl)-3-(pentadeutero-phenyl) propanamine L-tartrate) and 5-HM (N,N- diisopropyl-3-(2-hydroxy-5-hydroxymethylphenyl)-3-(pentadeutero-phenyl) 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 LC/MS/MS using Zorbax 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 serum concentration assay were linear over the range of 0.1-100 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%-3.3% and -3.5%-0.9%. Assay precision for tolterodine and 5-HM was 4.5%-7.4% and 4.6%-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 non-compartmental analysis using the Kinetica software (version 3.1; InnaPhase, PA, USA). 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 linear pharmacokinetics of tolterodine was confirmed up to 8 mg (Clemett and Jarvis, 2001).

Serum concentrations for the 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):

\[
\begin{align*}
\text{fu}_{\text{tolterodine}} & = 1 / (1+(2100 \times \text{AGP (g/L)}/ 42)) \\
\text{fu}_{\text{5-HM}} & = 1 / (1+(130 \times \text{AGP (g/L)}/ 42))
\end{align*}
\]

where \( \text{fu}_{\text{tolterodine}} \) and \( \text{fu}_{\text{5-HM}} \) are unbound fraction of tolterodine and 5-HM, respectively.

The concentration of the active moiety = (\( \text{fu}_{\text{tolterodine}} \) x serum concentration of tolterodine) + (\( \text{fu}_{\text{5-HM}} \) x serum concentration of 5-HM).

For the calculation of active moiety concentration, serum concentrations of tolterodine and 5-HM were converted to molar concentrations (nM) 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 biotransformed to 5-HM were estimated by the following steps:

(1) Estimation of intrinsic clearance of CYP2D6 for tolterodine and the amount of tolterodine biotransformed to 5-HM in each CYP2D6 genotype

Intrinsic clearance of tolterodine (CLint_{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 parallel tube model with assumption of ignorable renal clearance due to low urinary excretion (<3% (Brynne et al., 1998)) and fraction absorbed (fa) x intestinal availability (fg) equalled 1. The fa x fg for tolterodine was assumed to be almost 1 because it was reported that bioavailability of tolterodine in PM was 0.91 (Brynne et al., 1998; Van Kerrebroeck et al., 2001). Parallel tube model was used for the estimation because the mean of fu_{tolterodine} x intrinsic clearance of CYP2D6 for tolterodine (CLint_{CYP2D6,tolterodine}) in CYP2D6*1/*1 was estimated as 157 L/h, which was higher than hepatic blood flow rate (Qh: 91.8 L/h (Kato et al., 2003)) (Ito and Houston, 2004). The median CLint_{tolterodine} in 2 subjects of CYP2D6*4/*4 in the present study was used as the CLint of tolterodine by the metabolic pathway other than CYP2D6 (CLint_{other,tolterodine}). It was confirmed that the median CLint_{tolterodine} in CYP2D6*4/*4 in the present study (436 L/h) was consist with the estimation (443 L/h) from the total clearance in PM after intravenous infusion of tolterodine (Brynne 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 estimated individual CLint_{CYP2D6,tolterodine}. Dosage of
tolterodine was set at 2.74 mg as tolterodine, which was correspondent to 4 mg of tolterodine tartrate. The equations are shown in Appendix 1.

(2) Estimation of intrinsic clearance of CYP2D6 for 5-HM in each CYP2D6 genotype
With the estimated amount of 5-HM biotransformed from tolterodine calculated above, intrinsic clearance for 5-HM (CLint_{CYP2D6,5-HM}) in each CYP2D6 genotype was estimated from individual dose-normalized 5-HM AUC_{0-24}, individual fu_{5-HM} in the present study, reported renal clearance of 5-HM (Malhotra et al., 2008) and CLint of 5-HM by the metabolic pathway other than CYP2D6 (CLint_{other,5-HM}). The equations are shown in Appendix 2. Due to no data was available to calculate CLint_{other,5-HM} in the present study, CLint_{other,5-HM} was estimated from the pharmacokinetic parameters of 5-HM after intravenous infusion of 5-HM in EM and after oral administration of fesoterodine, which was a compound of esterified 5-HM, in EM and PM (http://www.emea.europa.eu/humandocs/Humans/EPAR/toviaz/toviaz.htm).

Simulation of the exposures of unbound tolterodine, 5-HM and active moiety with changing CLint_{CYP2D6}
Using the mean of estimated intrinsic clearances for tolterodine and for 5-HM in CYP2D6^{*1/*1} and CYP2D6^{*4/*4}, the changes of AUCs of unbound tolterodine and 5-HM with changing CLint_{CYP2D6} were simulated. Dosage of tolterodine was set at 2.74 mg as tolterodine, which was correspondent to 4 mg of tolterodine tartrate. The mean fu_{tolterodine} and fu_{5-HM}, which were 0.037 and 0.36 (Pahlman and Gozzi, 1999), and renal clearance of 5-HM (Malhotra et al., 2008) were used in this simulation. The reduction rate of CLint_{CYP2D6} was changed from no reduction (0%, EM) to 100% reduction equaled to the condition in PM. Active moiety exposure was calculated as the sum of AUCs of unbound tolterodine and 5-HM. Details of the simulation are shown in Appendix 3.
Statistical analysis

Statistical analysis for dose normalized Cmax and AUC$_{0-24}$ of tolterodine, 5-HM and active moiety among genotypes was performed by one-way analysis of variance (ANOVA) and Bonferroni post hoc test by using SAS (version 8.02, SAS Institute Inc., NC, USA). $CYP2D6^{*5/*10}$ and $CYP2D6^{*4/*4}$ were not included in the ANOVA because only 2 subjects were classified as each genotype. Differences were considered to be statistically significant when P values were less than 0.05. Additionally, simulation of the change of exposures was conducted using SAS.
Results

Genotyping
The allele frequencies of CYP2D6*10 in Asian and Caucasian subjects were 40% and 1.4%, respectively. The allele frequency of CYP2D6*5 was 5.6% in Asians and 1.4% in Caucasians. There were no Asian subjects who had CYP2D6*4 allele but the allele frequency of CYP2D6*4 in Caucasians 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 Figure 1, Figure 2 and Figure 3, respectively.
The mean values of dose-normalized Cmax and AUC0-24 of tolterodine in CYP2D6*1/*10 were similar to CYP2D6*1/*1, whereas those of CYP2D6*1/*4 or CYP2D6*1/*5 were approximately 1.9- and 2-fold higher than CYP2D6*1/*1 (Table 2).
Both Cmax and AUC0-24 in CYP2D6*10/*10 were significantly higher than CYP2D6*1/*1 and CYP2D6*1/*10 (Figure 1). They were approximately 2.6- and 3-fold higher than CYP2D6*1/*1, respectively. The Cmax and AUC0-24 values of 2 subjects of CYP2D6*5/*10 were higher than the mean value 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 of CYP2D6*1/*1, respectively. The values of CYP2D6*5/*10 and CYP2D6*4/*4 were not included in the 95% confidence interval (95%CI) of CYP2D6*10/*10 (Table 2).
The metabolite, 5-HM was unquantifiable in the subjects of CYP2D6*4/*4. In other genotypes, Cmax values were distributed within the same range (Figure 2). The mean values of AUC0-24 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/*10 was similar to CYP2D6*1/*1. The mean AUC\(_{0-24}\) values in CYP2D6*1/*4 or CYP2D6*1/*5 and CYP2D6*10/*10 were 1.2- and 1.5-fold higher than the value of 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 and they were not included in the 95% CI of CYP2D6*1/*1 (Table 2).

As active moiety, the mean values of Cmax and AUC\(_{0-24}\) 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 Cmax value in CYP2D6*10/*10 was 1.4-fold higher than CYP2D6*1/*1 and it was statistically significant (Figure 3). The mean AUC\(_{0-24}\) values in 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\(_{0-24}\) in CYP2D6*10/*10 was 1.8-fold higher than CYP2D6*1/*1 and was significantly higher than CYP2D6*1/*1 and CYP2D6*1/*10. The individual values of CYP2D6*5/*10 were included in the 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 metabolisms

CL\text{int}\textsubscript{CYP2D6} for tolterodine and 5-HM metabolisms in each genotype can be estimated from the AUC values using liver model. The estimated CL\text{int}\textsubscript{CYP2D6} for tolterodine and 5-HM metabolisms in each genotype are shown in Table 3.

CL\text{int}\textsubscript{CYP2D6,tolterodine} and CL\text{int}\textsubscript{CYP2D6,5-HM} in IMs (CYP2D6*1/*10, CYP2D6*1/*4 or
CYP2D6*1/*5, CYP2D6*10/*10 and CYP2D6*5/*10) were estimated to be lower than that in EM (CYP2D6*1/*1). Both for tolterodine and 5-HM, the relative enzyme activities to CYP2D6*1/*1 in CYP2D6*1/*10, CYP2D6*1/*4 or CYP2D6*1/*5 and CYP2D6*10/*10 were estimated as approximately 70%, 50% and 40% of CYP2D6*1/*1, respectively (Table 3). Individual relative activity in 2 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 of EM (CYP2D6*1/*1) and PM (CYP2D6*4/*4) are determined, the AUC in IM can be simulated by changing CLintCYP2D6 from EM to PM. The simulated AUCs of unbound tolterodine, unbound 5-HM and active moiety with changing CLintCYP2D6 are shown in Figure 4. The observed mean AUCs of each genotype in the present study were plotted at the reduction rate of CLintCYP2D6,tolterodine from CYP2D6*1/*1 (100% - relative activity in Table 3).

In the simulation, tolterodine AUC continued to increase from the exposure in EM (CYP2D6*1/*1) to the exposure in PM (CYP2D6*4/*4) with decreasing CLintCYP2D6 (Figure 4). The exposure increased steeply from about 80% reduction in CLintCYP2D6. For 5-HM, from the level in EM, AUC increased to the peak with decreasing enzyme activity around 80% reduction in CLintCYP2D6, and fell to zero. As active moiety, the sum of unbound fraction of tolterodine and 5-HM, the exposure described a curve with the peak around 90% reduction in CLintCYP2D6 and the exposure at the peak was 1.9-fold higher than EM. It was simulated that the exposures in EM and PM 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 EM and PM. The simulated AUC 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 $f_{u_{tolterodine}}$ and $f_{u_{5-HM}}$ for this simulation (see Method section). When $f_{u_{tolterodine}}$ and $f_{u_{5-HM}}$ are changed from minimum ($f_{u_{tolterodine}}$: 0.016 and $f_{u_{5-HM}}$: 0.21) to maximum (0.063 and 0.52, respectively) in the present study, the maximum increase of the active moiety from EM (2.3-fold) was similar to the result of mean $f_{u_{tolterodine}}$ and $f_{u_{5-HM}}$ (1.9-fold).
**Discussion**

This is the first report of clinical study to demonstrate the impact of *CYP2D6*\(^{*10}\) allele on tolterodine and 5-HM exposures. We also provided the first example of which IM shows higher exposure to pharmacological active moiety than EM and PM.

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*\(^{*21}\), *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*\(^{*1/*1}\) showed higher serum concentrations of tolterodine and lower concentration 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 in *CYP2D6*\(^{*1/*1}\) and homozygous of non-functional allele (*CYP2D6*\(^{*4/*4}\)) were comparable with those for EM and PM previously reported (Olsson and Szamosi, 2001). As a result, the active moiety exposures were almost the same between *CYP2D6*\(^{*1/*1}\) and *CYP2D6*\(^{*4/*4}\) in the present study (24 nM h in *CYP2D6*\(^{*1/*1}\) and 30 nM h in *CYP2D6*\(^{*4/*4}\)), as previously reported.

As expected from enzyme activity reduced by *CYP2D6*\(^{*10}\) *in vitro* studies (Yokota et al., 1993; Johansson et al., 1994), the exposure of tolterodine 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 5-HM exposure would be reversed. However, 5-HM AUC increased in the same order as tolterodine AUC except for CYP2D6*4/*4.

The active metabolite 5-HM is the metabolite of tolterodine by CYP2D6 and 5-HM itself is also metabolized by CYP2D6. When the activity of CYP2D6 reduces, 5-HM exposure may decrease due to decreasing the amount of 5-HM biotransformed from tolterodine (reduction of the exposure), whereas exposure of 5-HM may increase due to reduction of clearance for 5-HM (enhancement of the exposure) (Figure 5). The 5-HM exposure in subjects with reduced function allele depends on the balance of opposite directional effects, reduction and enhancement of 5-HM exposure.

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 of the present study. Those in CYP2D6*1/*10, CYP2D6*1/*4 or CYP2D6*1/*5 and CYP2D6*10/*10 were approximately 70%, 50% and 40% of CYP2D6*1/*1, respectively, both for tolterodine and 5-HM. Though Shen et al. reported that the extent of decrease in enzyme activity of CYP2D6*10 in comparison with CYP2D6*1 was different among various substrates in vitro (Shen et al., 2007), at least for tolterodine and 5-HM, it was expected that the relative enzyme activity of CYP2D6*10 compare to CYP2D6*1 were the same.

In the simulation with the estimated CLintCYP2D6, in contrast with continuous increase of tolterodine exposure with decreasing enzyme activity, the 5-HM exposure described a curve with a peak (Figure 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 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 at about 80% reduction in CLintCYP2D6, it was between 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, the 5-HM exposure fell to zero, but active moiety exposure was still higher than that in CYP2D6*1/*1 due to compensation by steep increase of 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 those in EM (CYP2D6*1/*1) and PM (CYP2D6*4/*4). This simulation result was well matched with observed phenomenon in the present study and the highest simulated active moiety exposure was around the observed value of CYP2D6*5/*10.

With regard to the Cmax of 5-HM, no differences were observed among genotypes although AUCs in IMs were higher than EM. This is possibly explained as follows: The formulation was sustained release formulation and the releasing of tolterodine could be considered as infusion. The 5-HM Cmax 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 Cmaxs were observed the same but AUCs were different among genotypes.

In the present study, as a result of a 3-fold increase of tolterodine exposure and 1.5-fold increase of 5-HM exposure, active moiety exposure which is the sum of unbound fraction of tolterodine and 5-HM was 1.8 fold in subjects with CYP2D6*10/*10 than CYP2D6*1/*1 and this was also higher than CYP2D6*4/*4 (Figure 3 and Table 2). In the simulation, the highest simulated active moiety exposure was around the observed value of CYP2D6*5/*10 and it was 1.9-fold higher than EM (CYP2D6*1/*1). Although such difference in exposure could cause the difference of optimal doses between Western and Asian countries, the approved Asian dosage and administration is the same as the US/EU, a once a daily dose of 4 mg tolterodine PR capsule.
For tolterodine case, difference of active moiety exposure between the ethnicities is presumably within the range of the safety and efficacy margins.

When only a parent compound has a significant pharmacological effect, the order of exposure to pharmacological active moiety is PM > IM > EM because the exposure continues to increase with decreasing enzyme activity. However, if a parent drug and its metabolite have a pharmacological effect and both of them are predominantly metabolized by CYP2D6, there is a possibility that IM has the highest active moiety exposure. In such case, Asians who predominantly possesses reduced function alleles has possibly higher exposure of the pharmacological active moiety than the populations who do not, in general, posses reduced function alleles, such as Caucasians. This should be taken into consideration for optimal dose selection in global drug development.
References


Footnotes

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Legend for figures

Figure 1 Dose normalized Cmax and AUC of tolterodine in each genotype. Left panel shows Cmax and right panel shows AUC$_{0-24}$. The box edges show 25th and 75th percentiles. The whiskers extend from each box edge to the furthest data within a distance of 1.5 interquantile ranges from 25th and 75th percentiles. The open circles outside the whiskers represent outliers. Significantly higher than CYP2D6*1/*1 (*), and higher than CYP2D6*1/*10 (†), using Bonferroni’s post hoc test (P<0.05).

Figure 2 Dose normalized Cmax and AUC of 5-HM in each genotype. Left panel shows Cmax and right panel shows AUC$_{0-24}$. The box edges show 25th and 75th percentiles. The whiskers extend from each box edge to the furthest data within a distance of 1.5 interquantile ranges from 25th and 75th percentiles. The open circles outside the whiskers represent outliers. Significantly higher than CYP2D6*1/*1 (*), and higher than CYP2D6*1/*10 (†), using Bonferroni’s post hoc test (P<0.05).

Figure 3  Dose normalized Cmax and AUC of active moiety in each genotype. Left panel shows Cmax and right panel shows AUC$_{0-24}$. The box edges show 25th and 75th percentiles. The whiskers extend from each box edge to the furthest data within a distance of 1.5 interquantile ranges from 25th and 75th percentiles. The open circles outside the whiskers represent outliers. Significantly higher than CYP2D6*1/*1 (*), and higher than CYP2D6*1/*10 (†), using Bonferroni’s post hoc test (P<0.05).

Figure 4 Relationships between AUCs of unbound fraction of tolterodine (green), 5-HM (blue) and active moiety (red) after tolterodine administration and reduction rate of CLint$_{CYP2D6}$ from CYP2D6*1/*1. Simulated AUCs of unbound tolterodine, unbound 5-HM and active moiety are shown in green, blue and red solid line, respectively. Mean values of observed AUC of unbound tolterodine, unbound 5-HM and active moiety in the present study in CYP2D6*5/*10 (■), CYP2D6*1/*10 (●), CYP2D6*1/*4 or *1/*5 (♦), CYP2D6*10/*10 (▲) are shown at the reduction rate of CLint$_{CYP2D6,tolterodine}$ from CYP2D6*1/*1 in each genotype (100% - relative activity in Table 3) as closed symbols with standard deviation. Individual values of CYP2D6*5/*10 and CYP2D6*4/*4 are shown as open triangles and open inverted triangles, respectively.

Figure 5 The relationships between change of CYP2D6 enzyme activity and exposure of tolterodine and 5-HM among EM, IM and PM. Square boxes are the compartments for tolterodine and 5-HM. Shaded areas in the boxes are the...
amount of each compounds in the compartment. Width of allows are correspondent to the contribution ratio of each clearance of metabolic/excretion pathway. Tolterodine exposure increases with decreasing CYP2D6 enzyme activity. 5-HM exposure in IM who has reduced CYP2D6 enzyme activity is determined by the balance between opposite directional effects, i.e. reduction and enhancement of the exposure. IM can have higher 5-HM exposure than EM when the enhancement effect is dominant. As a result, IM has the highest exposure of active moiety, sum of unbound tolterodine and 5-HM.
Table 1 Demographics of subjects in each genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1/*1</td>
<td>male</td>
<td>20 (44.4%)</td>
<td>32.9 (10.1)</td>
<td>166.7 (8.9)</td>
</tr>
<tr>
<td>(n=45)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/*10</td>
<td>male</td>
<td>6 (19.4%)</td>
<td>33.3 (9.6)</td>
<td>162.8 (8.7)</td>
</tr>
<tr>
<td>(n=31)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/*4 or *1/*5</td>
<td>male</td>
<td>3 (20.0%)</td>
<td>36.3 (10.2)</td>
<td>162.8 (8.0)</td>
</tr>
<tr>
<td>(n=15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*10/*10</td>
<td>male</td>
<td>5 (38.5%)</td>
<td>33.7 (10.1)</td>
<td>165.0 (7.7)</td>
</tr>
<tr>
<td>(n=13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*5/*10</td>
<td>male</td>
<td>1 (50.0%)</td>
<td>20, 43</td>
<td>150, 180</td>
</tr>
<tr>
<td>(n=2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*4/*4</td>
<td>male</td>
<td>1 (50.0%)</td>
<td>21, 25</td>
<td>160, 161</td>
</tr>
<tr>
<td>(n=2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean (standard deviation (SD)) except for gender. If number of subject is less than 2, individual values are shown instead of descriptive summary.
Table 2 Dose normalized Cmax and AUC of tolterodine, 5-HM and active moiety in each genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Tolterodine Cmax (ng/mL)</th>
<th>Tolterodine AUC₀–2₄ (ng h/mL)</th>
<th>5-HM Cmax (ng/mL)</th>
<th>5-HM AUC₀–2₄ (ng h/mL)</th>
<th>Active moiety Cmax (nM)</th>
<th>Active moiety AUC₀–2₄ (nM h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1/*1 (n=45)</td>
<td>1.65 (2.66)</td>
<td>17.05 (33.66)</td>
<td>2.09 (1.24)</td>
<td>21.39 (10.44)</td>
<td>2.35 (1.01)</td>
<td>24.23 (8.60)</td>
</tr>
<tr>
<td></td>
<td>[0.85 - 2.45]</td>
<td>[6.93 - 27.16]</td>
<td>[1.72 - 2.47]</td>
<td>[18.26 - 24.53]</td>
<td>[2.04 - 2.65]</td>
<td>[21.65 - 26.82]</td>
</tr>
<tr>
<td>*1/*10 (n=31)</td>
<td>1.64 (0.92)</td>
<td>17.18 (8.80)</td>
<td>2.11 (0.84)</td>
<td>24.18 (7.79)</td>
<td>2.74 (1.10)</td>
<td>31.39* (10.73)</td>
</tr>
<tr>
<td></td>
<td>[1.30 - 1.98]</td>
<td>[13.95 - 20.40]</td>
<td>[1.80 - 2.41]</td>
<td>[21.32 - 27.04]</td>
<td>[2.33 - 3.14]</td>
<td>[27.45 - 35.33]</td>
</tr>
<tr>
<td>*1/*4 or *1/*5 (n=15)</td>
<td>3.18 (2.08)</td>
<td>33.63 (29.04)</td>
<td>2.28 (1.05)</td>
<td>26.70 (7.50)</td>
<td>3.02 (1.24)</td>
<td>35.40* (9.55)</td>
</tr>
<tr>
<td></td>
<td>[2.03 - 4.33]</td>
<td>[17.54 - 49.71]</td>
<td>[1.70 - 2.85]</td>
<td>[22.54 - 30.85]</td>
<td>[2.33 - 3.71]</td>
<td>[30.11 - 40.70]</td>
</tr>
<tr>
<td>*10/*10 (n=13)</td>
<td>4.31* † (2.87)</td>
<td>51.83* † (36.26)</td>
<td>2.33 (0.87)</td>
<td>31.36* (10.57)</td>
<td>3.35* (1.51)</td>
<td>44.23* † (18.36)</td>
</tr>
<tr>
<td></td>
<td>[2.58 - 6.04]</td>
<td>[29.92 - 73.74]</td>
<td>[1.80 - 2.85]</td>
<td>[24.97 - 37.75]</td>
<td>[2.44 - 4.26]</td>
<td>[33.14 - 55.33]</td>
</tr>
<tr>
<td>*5/*10 (n=2)</td>
<td>6.06, 6.34</td>
<td>79.04, 87.09</td>
<td>1.89, 2.25</td>
<td>26.84, 32.24</td>
<td>2.89, 3.84</td>
<td>40.19, 54.92</td>
</tr>
<tr>
<td>*4/*4 (n=2)</td>
<td>17.82, 18.84</td>
<td>346.10, 358.94</td>
<td>NA</td>
<td>NA</td>
<td>1.52, 1.60</td>
<td>29.54, 30.64</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD) and [95% confidence interval]. If number of subject is less than 2, individual values are shown instead of descriptive summary. NA: not applicable.

Significantly higher than CYP2D6*1/*1 (*), and higher than CYP2D6*1/*10 (†), using Bonferroni’s post hoc test (P<0.05).
Table 3 Estimated intrinsic clearances of CYP2D6 for tolerodine and for 5-HM and the relative enzyme activity to CYP2D6*1/*1 in each genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Tolterodine</th>
<th>5-HM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CLint&lt;sub&gt;CYP2D6&lt;/sub&gt;, tolerodine (L/h)</td>
<td>Relative enzyme activity (%)</td>
</tr>
<tr>
<td>*1/*1</td>
<td>4441 (1479)</td>
<td>100</td>
</tr>
<tr>
<td>(n=45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/*10</td>
<td>2898 (1136)</td>
<td>65</td>
</tr>
<tr>
<td>(n=31)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/*4 or *1/*5</td>
<td>2121 (984)</td>
<td>48</td>
</tr>
<tr>
<td>(n=15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*10/*10</td>
<td>1594 (1287)</td>
<td>36</td>
</tr>
<tr>
<td>(n=13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*5/*10</td>
<td>435, 729</td>
<td>10, 16</td>
</tr>
<tr>
<td>(n=2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean (SD). If number of subject is less than 2, individual values are shown instead of descriptive summary.
Figure 1

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Figure 3

This article has not been copyedited and formatted. The final version may differ from this version.
Figure 4

AUC of unbound fraction (nM h)

Reduction rate of CLintCYP2D6 from *1/*1 (%)
Figure 5

Tolterodine

5-HM

Reduction of 5-HM Exposure
Decreasing the amount of 5-HM from tolterodine

Enhancement of 5-HM Exposure
Decreasing of 5-HM clearance
Appendix

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):

\[
CL_{\text{int,tolterodine}} = -\frac{Qh}{fu_{\text{tolterodine}}} \log e \left\{ \frac{Qh}{Dose \cdot fa \cdot fg} \right\} + \frac{Qh}{AUC_{0-24,\text{tolterodine}} \cdot Rb_{\text{tolterodine}}} 
\]

\[
CL_{\text{int,CYP2D6,tolterodine}} = CL_{\text{int,tolterodine}} - CL_{\text{int,other,tolterodine}}
\]

where \(Qh\): hepatic blood flow rate, \(fu\): fraction unbound, \(CL_{\text{int}}\): intrinsic clearance of each enzyme, \(fa\): fraction absorbed, \(fg\): intestinal availability. Subscript “other” means hepatic pathway other than CYP2D6 which is correspondent to hepatic pathway in PM.

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 body weight, as approximately equal to mean body weight of the present study. The \(fu\) of tolterodine (\(fu_{\text{tolterodine}}\)) was calculated from individual AGP concentrations as described in the method section. Clearances were adjusted by blood to serum concentration ratio (\(Rb_{\text{tolterodine}}\): 0.6). The median value of \(CL_{\text{int,tolterodine}}\) in \(CYP2D6^{*4/*4}\) in the present study, 436 L/h was used as \(CL_{\text{int,other,tolterodine}}\). Dose was set at 2.74 mg as tolterodine, which was correspondent 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 (\(Dose_{5\text{-HM}}\)) was calculated as follows:
where \( f_{m_{\text{CYP2D6, tolterodine}}} \) the ratio of contribution of CYP2D6 to the CYP-mediated tolterodine clearance, MW: molecular weight, those of tolterodine and 5-HM were 325.5 and 341.5, respectively.

Appendix 2. Equations for the estimation of intrinsic clearance of CYP2D6 for 5-HM in each CYP2D6 genotype (parallel tube model):

\[
D_{5-\text{HM}} = D_\text{o} \cdot f_a \cdot f_g \cdot f_{m_{\text{CYP2D6, tolterodine}}} \cdot \frac{MW_{\text{tolterodine}}}{MW_{5-\text{HM}}}
\]

\[
f_{m_{\text{CYP2D6, tolterodine}}} = \frac{CL \text{ int}_{\text{CYP2D6, tolterodine}}}{CL \text{ int}_{\text{tolterodine}}}
\]

\[
CL \text{ int}_{5-\text{HM}} = - \frac{Q_h}{fu_{5-\text{HM}}} \cdot \log_\epsilon \left( \frac{Q_h + CLR_{5-\text{HM}}}{Q_h + \left( \frac{D_{5-\text{HM}}}{AUC_{0-24,5-\text{HM}} \cdot R_{b_{5-\text{HM}}}} \right)} \right)
\]

\[
CL \text{ int}_{\text{other } 5-\text{HM}} = - \frac{Q_h}{fu_{5-\text{HM}}} \cdot \log_\epsilon \left( \frac{Q_h + CLR_{5-\text{HM}}}{Q_h + CLP_{\text{PM, fesoterodine}} \cdot f_a_{\text{fesoterodine}} \cdot f_g_{\text{fesoterodine}}} \right)
\]

\[
CL \text{ int}_{\text{CYP2D6, } 5-\text{HM}} = CL \text{ int}_{5-\text{HM}} - CL \text{ int}_{\text{other } 5-\text{HM}}
\]

where the fu of 5-HM (\( fu_{5-\text{HM}} \)) was calculated from individual AGP concentrations as described in the method section. Renal clearance (CLR) was 16.4 L/h (Malhotra et al., 2008). Clearances were adjusted by blood to serum concentration ratio (\( R_{b_{5-\text{HM}}} \); 0.8).

The intrinsic clearance for 5-HM by the metabolic pathway other than CYP2D6 was calculated as 60.99 L/h from oral clearance of 5-HM after administration of fesoterodine in PM (CLP_{\text{PM, fesoterodine}}). This was calculated from AUC after 4 mg dose of fesoterodine tablet, which was 44.5 ng h/mL (Malhotra et al., 2008). The \( f_a_{\text{fesoterodine}} \times f_g_{\text{fesoterodine}} \) was calculated as 0.70 from reported bioavailability of 5-HM in EM (\( f_a_{\text{fesoterodine}} \times f_g_{\text{fesoterodine}} \times f_{h_{5-\text{HM, EM}}} \)) which was 42% as the ratio of 5-HM AUC after oral administration of fesoterodine tablet vs 5-HM IV dose in EM.
(http://www.emea.europa.eu/humandocs/Humans/EPAR/toviaz/toviaz.htm) and total clearance after administration of 5-HM IV dose in EM. Molecular weight of fesoterodine fumarate and 5-HM were 527.5 and 341.5, respectively.

Appendix 3. Simulation of the exposures of unbound tolterodine, 5-HM and active moiety with changing intrinsic clearance of CYP2D6

\[
AUC_{\text{tolterodine,free}} = \frac{Dose \cdot fa \cdot fg \cdot \exp(-fu_{\text{tolterodine}} \cdot CL_{\text{intCP2D6,tolterodine}} + CL_{\text{intother,tolterodine}})}{Qh \left( 1 - \exp(-fu_{\text{tolterodine}} \cdot \frac{CL_{\text{intCP2D6,tolterodine}} + CL_{\text{intother,tolterodine}}}{Qh} \right)}
\]

\[
AUC_{5-HM,free} = \frac{Dose \cdot fa \cdot fg \cdot \exp(-fu_{5-HM} \cdot \frac{CL_{\text{intCP2D6,5-HM}} + CL_{\text{intother,5-HM}}}{Qh})}{Qh \left( 1 - \exp(-fu_{5-HM} \cdot \frac{CL_{\text{intCP2D6,5-HM}} + CL_{\text{intother,5-HM}}}{Qh} \right) + CLR_{5-HM}}
\]

where \( CL_{\text{intCP2D6,tolterodine}} \) and \( CL_{\text{intCP2D6,5-HM}} \) were changed from no reduction which was the mean value of \( CYP2D6^{*1/*1} \) in the present study (4441 L/h for tolterodine and 130.6 L/h for 5-HM, respectively) to 100% reduction equaled to the condition in PM. For all other parameters, the same values in Appendix 1 and 2 were used for the simulation. Active moiety exposure was calculated as the sum of molar AUCs of unbound tolterodine and 5-HM.