Pharmacokinetics of Tolterodine in Japanese and Koreans: Physiological and Stochastic Assessment of Ethnic Differences

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Summary: Tolterodine is known as a drug which exhibits ethnic differences in pharmacokinetics between Japanese and Koreans despite genetic similarities among the populations of East Asian countries. Tolterodine is mainly metabolized by CYP2D6 to a 5-hydroxymethyl metabolite (5-HM), and 5-HM is also metabolized by CYP2D6. The reduced-function allele CYP2D6*10 is frequently observed in Asian populations. We investigated differences in the pharmacokinetics of tolterodine between small Japanese and Korean study populations by physiological and stochastic approaches with consideration of the CYP2D6 genotype. The genotype frequencies of CYP2D6*10/*10 and CYP2D6*5/*10 were found to be higher in Koreans than in Japanese, which suggested that this frequency difference occurred incidentally. The effects of CYP2D6 genotype and ethnicity on the intrinsic clearance of tolterodine by CYP2D6 were tested and only genotype was found to be a significant factor by ANCOVA. A simulation was conducted to confirm whether the observed differences in tolterodine exposure could be explained by the differences in genotype frequency found in this study. It was confirmed that the variability of intrinsic clearance could be responsible for the incidental exposure differences. In conclusion, apparent differences in exposure were found between small Japanese and Korean study populations because of the variability of intrinsic clearances and genotype frequencies.

Keywords: drug metabolism; Japanese; Korean; cytochrome P450 2D6; ethnicity; genotype; phenotype; regulatory science

Introduction

Ethnic differences represent one of the most important factors in global clinical trials (GCTs). The effect of ethnicity should carefully be assessed in GCTs because of ethnicity-related differences in drug efficacy and safety.11 Ichimaru et al. stated that GCTs conducted only in East Asian countries could generate more consistent results than studies that include other populations outside East Asia11 since genetic similarities in East Asian countries are well known.2,3 However, they pointed to tolterodine as a drug having possible pharmacokinetic differences among populations of East Asian countries41 and emphasized the importance of further investigation on the effect of ethnicity even within the same region.

Tolterodine is a drug for treatment of over-active bladder and was approved in Japan as a result of a single Japanese-Korean Asian study.41 Tolterodine is mainly metabolized by CYP2D6 to a pharmacologically active 5-hydroxymethyl metabolite (5-HM),5,6 and 5-HM is also metabolized by two major pathways by CYP2D6 and CYP3A4 and it is also renally excreted.6 The genetic polymorphism of CYP2D6 is well known,7 and the reduced-function allele CYP2D6*10 is frequently observed in Asian populations, although the frequency is negligible in other populations.8-11 The effect of CYP2D6*10 on tolterodine pharmacokinetics has already been investigated; it was confirmed that tolterodine exposure increased with enzyme activity reduction and that 5-HM exposure depended on the balance of opposing effects of decreasing enzyme activity, i.e., reduction and enhancement of exposure.12 It has already been reported that the genotype frequencies of CYP2D6 in Japanese and Koreans are the same.31 When this genetic similarity was taken into account, it was not expected that there would be a difference
in the pharmacokinetics of tolterodine between Japanese and Koreans; however, such a difference was nonetheless observed.4)

In this article, we investigated the causes of the observed differences in the pharmacokinetics of tolterodine between Japanese and Koreans using both physiological and stochastic approaches with the data of the pharmacokinetic study to which Ichimaru et al. referred.1) This is the first publication of an investigation into the differences in the pharmacokinetics of tolterodine between Japanese and Koreans.

Methods

Subjects and study design: Thirty-six (36) healthy Japanese volunteers (13 male and 23 female) and 36 healthy Korean volunteers (11 male and 25 female) were enrolled in a randomized, multiple-dose study. Korean subjects were matched to Japanese subjects for age (± 5 years) and body weight (± 10%). There were three dose groups (2, 4, and 6 mg of tolterodine l-tartrate) with 12 Japanese and 12 Koreans in each group. Each subject received single daily doses of tolterodine as prolonged release capsules for 5 consecutive days. Continuous use of any other medication was not permitted. The study was performed in accordance with the Declaration of Helsinki, ethical approval having been obtained from the local Institutional Review Board of the Health and Regulatory Affairs Authority. All volunteers gave their written informed consent prior to commencement of the study.

CYP2D6 genotyping: The genotyping method was described in a previous publication.12) In brief, the presence of CYP2D6*3, CYP2D6*4, CYP2D6*5, and CYP2D6*10 was tested in each subject using whole blood before the first dose by multiplex polymerase chain reaction (CYP2D6*3 and *4), long-range polymerase chain reaction (CYP2D6*5), and allele-specific amplification (CYP2D6*10). If no variations were detected on an allele, it was determined as the wild-type (CYP2D6*1). CYP2D6*3 and CYP2D6*4 were not detected in any Japanese or Korean subject.

Pharmacokinetic sampling: Steady-state venous blood samples (7 mL) for determination of tolterodine and 5-HM were collected at 0, 0.5, 1, 2, 3, 4, 6, 9, 12, 18, and 24 h after the last administration. A blood sample in 5-HM were collected at 0, 0.5, 1, 2, 3, 4, 6, 9, 12, 18, and 24 h after the last administration. A blood sample in

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).10) Assay accuracy for tolterodine and 5-HM was 0.2%–3.3% and −3.5% to −0.9%, respectively, and assay precision for tolterodine and 5-HM was 4.5%–7.4% and 4.6%–6.9%, respectively.

Pharmacokinetic analysis: Pharmacokinetic parameters were calculated by non-compartmental analysis using Kinetica software (version 3.1; InnaPhase, PA, USA). Maximum serum concentration (Cmax) and area under the concentration–time curve from 0 to 24 h after the last dose of multiple dosing (AUC) of tolterodine and 5-HM were dose normalized to the approved recommended daily dose of 4 mg. Individual intrinsic clearances of tolterodine by CYP2D6 (CLintCYP2D6,tolterodine) and of 5-HM by CYP2D6 (CLintCYP2D6,5-HM) were calculated using individual values of AUC and AGP concentration in the study, as described in a previous publication.12)

Simulation of tolterodine exposure based on the observed genotype frequency: A total of 1,000 sets of CLintCYP2D6,tolterodine values for 36 virtual Japanese and Korean subjects with the genotype frequencies found in this study were generated by Monte Carlo simulation using geometric mean (CV%) values of CLintCYP2D6,tolterodine for CYP2D6*1/*1, CYP2D6*1/*10, CYP2D6*1/*4 and CYP2D6*10/*10 of 3959 (64.2%), 2696 (38.8%), 1839 (62.6%) and 1187 (84.4%) L/h, respectively. For CYP2D6*5/*10, a median exposure of 582 L/h and the average CV of other genotypes (60%) were used because the CV could not be calculated for 2 subjects. Tolterodine AUC was generated from the simulated CLintCYP2D6,tolterodine values based on the parallel tube model as described in a previous publication.12) The simulation was conducted using SAS (version 9.02; SAS Institute Inc., Cary, NC).

Statistical analysis: Analysis of covariance (ANCOVA) with CYP2D6 genotype and ethnicity (Japanese and Korean) as fixed effects on AUCs and intrinsic clearances of tolterodine and 5-HM by CYP2D6 was performed by SAS (version 9.02; SAS Institute Inc., Cary, NC). Interaction between genotype and ethnicity was not included in the model because it was confirmed that there was no significant interaction. The proportion of the variance due to each factor was calculated as the proportion of total variance (σ2) by the method provided by Olejnik et al.13)

Results

Demographics and genotype frequency: Thirty-six healthy Japanese volunteers and 36 healthy Korean volunteers were enrolled in the study. The demographics of subjects are summarized in Table 1, and the allele and genotype frequencies of CYP2D6 in Japanese and Korean subjects are shown in Figure 1. Gender, age, height and

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>Gender (male: 36)</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japanese</td>
<td>13 (36.1%)</td>
<td>33.5</td>
<td>164.6</td>
<td>58.3</td>
</tr>
<tr>
<td>Korean</td>
<td>11 (30.6%)</td>
<td>33.6</td>
<td>163.5</td>
<td>58.8</td>
</tr>
</tbody>
</table>

Data are presented as mean (standard deviation (SD)) except for gender.
body weight of Japanese and Korean subjects were the same (Table 1). The most common mutation was CYP2D6*10 both in Japanese and Korean groups (Fig. 1a). The frequency of the CYP2D6*1/*10 genotype was about 40% in both ethnic groups and this was the most frequent genotype (Fig. 1b). The frequency of CYP2D6*1/*10 in Japanese was 11%, which was lower than that in Koreans (25%). Two Koreans were genotyped as CYP2D6*5/*10, whereas no Japanese subject had this genotype. The numbers of Japanese and Korean subjects in each genotype were within the 95% confidence intervals (CI) of binominal distribution calculated from the frequency in Japanese and Koreans reported by Myland et al. The 95% CI of the number of subjects in CYP2D6*1/*1, CYP2D6*1/*10, CYP2D6*1/*5, CYP2D6*10/*10 and CYP2D6*5/*10 were calculated as 6–16, 9–19, 0–4, 3–11 and 0–4, respectively.

**Tolterodine and 5-HM exposures:** The individual AUC values of tolterodine and 5-HM are plotted in Figure 2; the results of ANCOVA to investigate the effect of genotype and ethnicity on AUC are shown in Table 2.

The geometric mean of tolterodine AUC in Koreans was approximately twice that in Japanese (Fig. 2a). The difference between Japanese and Koreans tended to disappear in the comparison within each genotype. ANCOVA showed that both genotype and ethnicity were significant factors (p < 0.05) and the proportion of the variance due to genotype (27.8%) was approximately 5 times that of ethnicity (4.9%) (Table 2). In addition, the geometric mean of tolterodine Cmax was also 70% higher in Koreans than in Japanese, with the geometric means (CV%) of Japanese and Koreans being 1.27 (73.7%) and 2.15 (78.5%) ng/mL, respectively. The proportions of variance for genotype and ethnicity were 22.5% and 3.3%, and both of these were statistically significant (p < 0.05) according to ANCOVA.

The geometric mean of 5-HM AUC was 40% higher in Koreans than in Japanese (Fig. 2b). AUCs classified by genotype increased with expected enzyme activity reduction.
Statistically significant differences remained between Japanese and Koreans even due to mutations both in Japanese and Koreans. However, a difference remained between Japanese and Koreans even within each genotype. ANCOVA showed that both genotype and ethnicity were significant factors (p < 0.05), and the proportions of the variance due to genotype and ethnicity were both about 10% (Table 2). The geometric mean of 5-HM Cmax was also 30% higher in Koreans than in Japanese, the geometric means (CV%) being 2.20 (43.1%) and 1.66 (38.3%) ng/mL, respectively. The proportions of variance of genotype and ethnicity were 7.6% and <1%, and only genotype was statistically significant (p < 0.05) according to ANCOVA.

Intrinsic clearance of CYP2D6 for tolterodine and 5-HM: As a physiological approach, the influences of genotype and ethnicity on CLint_CYP2D6, tolterodine and CLint_CYP2D6, 5-HM were investigated. CLint_CYP2D6, tolterodine and CLint_CYP2D6, 5-HM in Japanese and Korean subjects are plotted in Figure 3a and Figure 3b, respectively. The results of ANCOVA are shown in Table 3.

The ratio of the geometric means (90% CI) of CLint_CYP2D6, tolterodine of Koreans to Japanese was 65.2% (48.9%–86.9%) (Fig. 3a); however, the difference disappeared within each genotype. The ratios of the geometric means (90% CI) of Koreans to Japanese for CYP2D6*1/*1, CYP2D6*1/*10 and CYP2D6*10/*10 were 100.6% (55.6%–181.8%), 81.3% (63.9%–103.5%) and 42.5% (18.5%–

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**Table 2. Results of ANCOVA on AUCs of tolterodine and 5-HM in healthy Japanese and Korean subjects (ng h/mL, n = 36 in each ethnic group)**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Ethnicity</th>
<th>Geometric mean (CV%)</th>
<th>Geometric mean (90% CI)</th>
<th>Proportion of variance (cv%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolterodine</td>
<td>Japanese</td>
<td>12.5 (83.9)</td>
<td>191.9 [118.1–217.1]</td>
<td>Genotype: 27.8%*</td>
</tr>
<tr>
<td></td>
<td>Korean</td>
<td>24.0 (80.2)</td>
<td>139.1–264.9 [NC]</td>
<td>Ethnicity: 4.9%*</td>
</tr>
<tr>
<td>5-HM</td>
<td>Japanese</td>
<td>18.8 (39.4)</td>
<td>143.6 [19.3–113.6]</td>
<td>Genotype: 11.1%*</td>
</tr>
<tr>
<td></td>
<td>Korean</td>
<td>27.0 (32.7)</td>
<td>124.6–165.6 [NC]</td>
<td>Ethnicity: 12.9%*</td>
</tr>
</tbody>
</table>

*Statistically significant by ANCOVA (p < 0.05).
97.7%), respectively. The geometric means for CYP2D6 *10/*10 appeared to be different, but the values of the 4 Japanese subjects were included in the range of the 9 Koreans. The individual values of the 4 Japanese and 2 Korean subjects for CYP2D6 *1/*5 were distributed in the same range. The values of the 2 Koreans for CYP2D6 *5/*10 were lower than the geometric mean for CYP2D6 *10/*10.

According to ANCOVA, only genotype was a significant factor contributing to the variance of CLint_{CYP2D6,tolterodine} (p < 0.05, Table 3). The proportion of variance due to genotype was 28.7%, whereas that due to ethnicity was only 1.1% and was not statistically significant (p = 0.14).

The ratio of geometric means (90% CI) of CLint_{CYP2D6,5-HM} of Koreans to Japanese was 54.2% (40.8–71.8%) (Fig. 3b). After classification by genotype, CLint_{CYP2D6,5-HM} decreased with expected enzyme activity reduction both in Japanese and Korean subjects. According to ANCOVA, both genotype and ethnicity were found to be significant factors contributing to variance in CLint_{CYP2D6,5-HM} (p < 0.05, Table 3). The proportion of variance due to genotype was 18.5%, which was 60% larger than that of ethnicity (11.3%).

Table 3. The result of ANCOVA on CLint_{CYP2D6,tolterodine} and CLint_{CYP2D6,5-HM} in healthy Japanese and Korean subjects (L/h, n = 36 in each ethnic group)

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Geometric mean (CV%)</th>
<th>Proportion of variance (CV%)</th>
<th>Genotype</th>
<th>Ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolterodine</td>
<td></td>
<td></td>
<td>Genotype</td>
<td>Ethnicity</td>
</tr>
<tr>
<td>Japanese</td>
<td>3020 (65.5)</td>
<td>65.2</td>
<td>Genotype: 28.7%*</td>
<td>Ethnicity: 1.1%</td>
</tr>
<tr>
<td>Korean</td>
<td>1969 (80.3)</td>
<td>(48.9–86.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HM</td>
<td></td>
<td></td>
<td>Genotype: 18.5%*</td>
<td>Ethnicity: 11.3%</td>
</tr>
<tr>
<td>Japanese</td>
<td>107.2 (48.5)</td>
<td>54.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Korean</td>
<td>58.1 (87.7)</td>
<td>(40.8–71.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Statistically significant by ANCOVA (p < 0.05).

Simulation of tolterodine exposure based on the observed genotype frequencies in the study: As a stochastic approach to confirm if the twofold difference in tolterodine exposure between Japanese and Korean subjects could be explained by the difference of genotype frequency in the present study, a simulation of tolterodine exposure was conducted. A total of 1,000 sets of tolterodine AUCs of 36 virtual Japanese and Korean subjects were generated using CLint_{CYP2D6,tolterodine} of each genotype and the observed genotype frequency in this study. The distribution of geometric means of simulated tolterodine AUCs of Japanese and Koreans is shown in Figure 4.

The medians of the simulated AUCs based on the observed genotype frequency in the Japanese and Korean groups were 14.8 and 19.1 ng h/mL, respectively (Fig. 4). The observed geometric means of the Japanese and Korean groups in the study, which were 12.5 and 24.0 ng h/mL, respectively, were included in the 95% CI of the simulated results (Japanese: 9.0–21.6 ng h/mL, Korean: 9.9–30.6 ng h/mL).

Fig. 4. Distribution of geometric means of simulated tolterodine AUCs based on the observed genotype frequencies of the Japanese and Korean groups in the present study

Blue and red curves indicate the Japanese and Korean groups, respectively. Vertical lines indicate observed geometric means of the Japanese and Korean groups in the present study, and shaded areas indicate 95% confidence intervals of the two groups.

Discussion

In the present study, the genotype frequencies in the Korean group of CYP2D6 *10/*10 and CYP2D6 *5/*10, which result in reduced intrinsic clearance by CYP2D6, (2) were higher than those in the Japanese group. It had already been reported that there are no genotype frequency differences between Japanese and Koreans. (3) The number of subjects in each genotype in the Japanese and Korean groups in this study was included in the 95% CI based on the genotype frequency reported by Myrand et al. (3) Therefore, it was considered that the apparent genotype frequency difference between the Japanese and Korean groups in this study could be the result of the small sample size of 36.

In this study, the geometric mean of tolterodine exposure of the Korean group was about twice that of the Japanese group; however, the difference in AUC between the groups tended to disappear within the same genotype. According to ANCOVA, genotype was selected as a significant and dominant factor to explain the variability of AUC. Ethnicity was also a significant factor, although the contribution was less. For 5-HM, AUC was 40% higher in the Korean group than in the Japanese group, and ANCOVA showed that both genotype and ethnicity were significant factors with respect to variability.

In theory, genotype is considered to have a direct effect on intrinsic clearance. It is well known that the nonlinear relationships between intrinsic clearance and hepatic clearance are described by mathematical models such as the parallel tube and dispersion models. Thus, exposure
changes nonlinearly with intrinsic clearance. In addition, the relationship between 5-HM exposure and CYP2D6 enzyme activity reduction is complicated because it depends on the balance of opposing effects of reduced enzyme activity, decreasing the amount of 5-HM biotransformed from tolterodine (reduction of the exposure) and decreasing clearance for 5-HM (enhancement of the exposure). \(^{12}\)

This physiological condition was taken into account and the effects of genotype and ethnicity on CL\textsubscript{int}\textsubscript{CYP2D6,tolterodine} were investigated. As a result, the effect of genotype on CL\textsubscript{int}\textsubscript{CYP2D6,tolterodine} was detected more clearly than that on AUC, whereas ethnicity was not selected as a significant factor of variability in CL\textsubscript{int}\textsubscript{CYP2D6,tolterodine}. Therefore, it was confirmed that tolterodine exposure differences between the Japanese and Korean groups was attributed to the differences in genotype frequency in the study, specifically those of CYP2D6*10/*10 and CYP2D6*5/*10. It is also considered that the ANCOVA results for AUC might be biased and that intrinsic clearance might be a more appropriate parameter to assess the influence of genotype and ethnicity. For 5-HM, as for tolterodine, the contribution of ethnicity to variability in CL\textsubscript{int}\textsubscript{CYP2D6,5-HM} was less than that of genotype. It was considered that the effect of genotype could be assessed more precisely by taking the physiological approach than by considering the AUC by taking the physiological approach. However, the effect of ethnicity still remained as a significant factor according to ANCOVA. The contribution of CYP2D6 to total clearance of tolterodine is about 90%, whereas that of 5-HM is only about 40% (data not shown), i.e., other metabolic pathways such as CYP3A4 and renal excretion are responsible for the remaining clearance. This presumably was reflected in the remaining effect of ethnicity.

For typical CYP2D6 substrate tolterodine, with about 90% CYP2D6 contribution to total clearance, we conducted a simulation to confirm if the twofold difference in tolterodine exposure between Korean and Japanese subjects could be explained by genotype frequency differences in the present study. As a result, it was confirmed that this difference could occur with consideration of the variability of the intrinsic clearance. This simulation indicated that apparent ethnic differences could be detected by chance even between the same populations when the sample size is small.

In general, the pharmacokinetics in different ethnic groups are compared in phase 1 studies with small numbers of subjects. However, in a small study, incidental sampling bias with respect to variability of genotype frequency and intrinsic clearances could occur, and this may lead to a misjudgment about ethnic difference(s). This should be considered when assessing the effect of ethnicity on pharmacokinetics in small-scale studies.

References


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