Sex-Dependent Differences in Cytochrome P450 3A Activity as Assessed by Midazolam Disposition in Humans: A Meta-Analysis

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Received November 20, 2009; accepted February 17, 2010

ABSTRACT:

Controversy exists concerning the sex-dependent differences in cytochrome P450 3A activity in humans. Meta-analysis of selected studies may address this question. Meta-analysis was performed on published or unpublished data in terms of sex-dependent differences in midazolam (MDZ) disposition in humans. The following pharmacokinetic parameters were included for the analysis: MDZ oral and systemic clearance, area under the concentration-time curve (AUC) of oral and intravenous MDZ, MDZ oral bioavailability (F), and MDZ gastrointestinal extraction (Eg). Ten studies including 409 healthy volunteers were identified. Women exhibited 16% higher weight-corrected MDZ oral clearance (P < 0.001) and 20% higher systemic clearance (P = 0.002) than men. No significant difference in the AUC after oral dosing of MDZ was noted between sexes. Women showed lower AUC of intravenous MDZ than men (P = 0.02). No sex-dependent differences were observed in F and Eg. In conclusion, women showed significantly greater hepatic CYP3A activity than men, whereas no sex-dependent difference in intestinal CYP3A activity was observed.

The enzyme subfamily cytochrome P450 3A and its two major members—CYP3A4 and, to a lesser extent, CYP3A5—are considered the most important drug-metabolizing enzymes by virtue of their high concentration, strategic localization, and wide substrate specificity (Thummel and Wilkinson, 1998).

Midazolam (MDZ) is a selective substrate of CYP3A4 and CYP3A5 and is almost exclusively metabolized by CYP3A to form the primary metabolite 1-hydroxymidazolam. Systemic or apparent oral clearance of MDZ is the most widely tested and accepted biomarker for hepatic and intestinal CYP3A activity (Streetman et al., 2000). Intravenous administration of MDZ reflects only hepatic CYP3A activity, whereas orally administered MDZ is a measure of intestinal and hepatic CYP3A activities (Thummel et al., 1996; Gorski et al., 1998; Streetman et al., 2000).

Numerous studies have examined potential sex differences in the metabolic activity of CYP3A. These studies include prospective clinical studies and retrospective analysis of data from previous studies. The findings from clinical studies have been inconsistent or inconclusive. For example, mean MDZ clearances were greater in women in several investigations, reaching statistical significance in some (Greenblatt et al., 1984; Gorski et al., 1998, 1999; Zhu et al., 2003; Chen et al., 2006a) or even insignificantly higher clearances in men (Thummel et al., 1996). Results of the largest-scale investigation (41 women and 58 men) showed that systemic clearances were 30% greater in women, but there was no sex difference for oral MDZ (Kharasch et al., 2007).

There were two retrospective studies on this topic, and both studies showed significant sex-dependent differences (Chen et al., 2006b; Miao et al., 2009). Miao et al. (2009) concluded that women exhibited 19% higher weight-corrected systemic clearance and 38% higher oral clearance than men (P < 0.05). This study reviewed the data from seven clinical studies, which were previously conducted by their research team, but only included subjects whose DNA samples were available. In addition, this study included a study conducted exclusively on women (without male volunteers) (Gorski et al., 2000). The oral clearance data of this study were surprisingly higher than those of other included studies. As a result, the retrospective study by Miao et al. (2009) is at a higher risk of bias compared with that for a prospective study. In the other retrospective study, Chen et al. (2006b) found that women exhibited 11% higher mean weight-corrected MDZ systemic clearance and 28% higher oral clearance than men (P ≤ 0.01). The authors of this retrospective study concluded that women showed significantly greater mean hepatic and intestinal CYP3A activity than men. However, many studies did not find a sex-dependent difference in MDZ oral bioavailability (Thummel et al., 1996; Gorski et al., 1998, 2003; Kharasch et al., 2007). As a result, further studies are needed to address the question of whether sex-dependent differences exist in the intestine.


Article, publication date, and citation information can be found at http://dmd.aspetjournals.org.

The online version of this article (available at http://dmd.aspetjournals.org) contains supplemental material.

ABBREVIATIONS: MDZ, midazolam; OM, AIC, area under the curve; F, MDZ oral bioavailability; Eg, gastrointestinal extraction; WMD, weighted mean difference; SMD, standardized mean difference; 95% CI, 95% confidence intervals; CIreg, weight-corrected midazolam oral clearance; Clsys, weight-corrected MDZ systemic clearance; AUCpo, AUC of oral MDZ; AUCiv, area under the concentration-time curve of intravenous MDZ.
In this meta-analysis, we quantitatively summarized the sex-dependent differences in CYP3A activity as assessed by MDZ disposition in humans. We also attempted to address the question of whether sex-dependent differences exist in the intestine.

Materials and Methods

Search Strategy. The electronic databases Ovid, Medline, PubMed, Embase, and The Cochrane Library 2009 (Issue 3) were searched from 1980 until September 2009. The search terms were midazolam, CYP3A, and sex (or gender). Two reviewers (W. Jia and Y.-S.Z.) screened reference lists from all articles and reviews retrieved to identify additional studies. Studies included were hand-searched to identify those that fit the selection criteria. We independently assessed these articles based on the inclusion criteria. Data derived from abstracts were also used. Any discrepancies about inclusion between the two researchers were resolved through consensus.

Inclusion Criteria. Studies were included if they were clinical trials from which the data of sex-dependent differences in MDZ disposition could be obtained. Studies were excluded if the study design showed different recruiting criteria for women and men, unless the difference in recruiting criterion was judged to have no influence on the outcome. Excluded subjects include patients or healthy volunteers who were taking medications known to affect CYP3A activity before study enrollment. Studies conducted on elderly volunteers were included because there was no significant difference in CYP3A activity between young and old populations (Klotz, 2009).

According to the retrospective studies by Miao et al. (2009) and Chen et al. (2006b), the sex difference in MDZ clearance ranged from 11 to 38%. Many relevant studies on this topic used a small sample size. To assure enough subjects to be included in our meta-analysis, we chose a conservative threshold (40% sex difference) for the estimation of sample size. Furthermore, this conservative threshold reduces the possibility of excluding small-sized studies of good methodology quality (potentially confounding factors were equally distributed between sexes). With use of MDZ clearance data (Shargel et al., 2006), we calculated the mean and S.D. of sex differences in CYP3A activity for 15 clinical studies included (Zhu et al., 2003). However, only selected studies eligible, three ineligible because of inadequate sample size, and one study unknown. The study protocol of the unpub-

Evaluating the Quality of the Eligible Studies. We also attempted to address the question of whether sex-dependent differences exist in the intestine. We also attempted to address the question of whether sex-dependent differences exist in the intestine.

Results

Study Characteristics and Methodological Quality. Twenty-seven potentially relevant studies were evaluated (Fig. 1 shows the numbers of studies evaluated at each stage). Nine clinical trials including 291 healthy volunteers and one retrospective study including 118 healthy volunteers were identified. Table 1 shows characteristics of the studies. Two of the clinical studies included were conducted in our own laboratory (Hu et al., 2009) (Z.-Y. Hu, Q. Yu, and Z.-N. Cheng, unpublished data). The study protocol of the unpublished study conformed to the ethical guidelines of the Declaration of Helsinki of 1975 (as revised in 1983) and was approved by the ethics committee of the School of Pharmaceutical Sciences, Central South
University. All subjects provided written informed consent before study procedures. Details of the unpublished study are provided as supplemental data.

In one included study (Kharasch et al., 2007), subjects were CYP3A5-genotyped before enrollment in the dosing phase of the investigation to ensure that there were enough subjects carrying at least one CYP3A5*1 allele. However, this study design may not affect the outcome of sex-dependent differences in MDZ clearance because the CYP3A5 genotype had no effect on the oral or systemic clearance of MDZ (Kharasch et al., 2007). In addition, gender is balanced between CYP3A5*1 carriers and noncarriers (Kharasch et al., 2007). A few female volunteers in two of the studies were taking oral contraceptives (Gorski et al., 1998, 2003). However, the original investigators previously demonstrated that oral contraceptives did not alter oral or systemic disposition of MDZ (Belle et al., 2002). Most of the studies included were crossover or fixed-order design (Table 1). Among these studies, seven of the studies also investigated the effect of CYP3A inhibitors (clarithromycin, ketoconazole, and fluvoxamine) or an inducer (rifampin) on MDZ disposition. The studies by Chen et al. (2006a,c) were already included in the retrospective study (Chen et al., 2006b). As a result, both studies were excluded from our meta-analysis.

There was no evidence of publication bias from either the funnel plot (refer to Supplemental Fig. S1) and Egger test (Table 2). The meta-analysis is summarized in Table 2.

### MDZ Oral Clearance

MDZ oral clearance was provided in seven published studies (Thummel et al., 1996; Gorski et al., 1998, 1999, 2003; Chen et al., 2006b; Kharasch et al., 2007; Hu et al., 2009) and an unpublished study (Z.-Y. Hu, Q. Yu, and Z.-N. Cheng, unpublished data) that enrolled 307 healthy volunteers. The meta-analysis showed significantly higher MDZ oral clearance in women than in men [WMD 0.20 (95% CI 0.11–0.28) l/h/kg; P < 0.001] (Fig. 2). Heterogeneity was not significant among these studies (I² = 41%, P = 0.10), so a fixed-effects model was used.

There was no change in result [WMD 0.26 (95% CI 0.13–0.38) l/h/kg; P < 0.001] if we excluded the study by Chen et al. (2006b) (retrospective study, weight = 57.7%) from the meta-analysis. The heterogeneity completely disappeared after the study of Kharasch et al. (2007) was excluded from the analysis (I² = 3%, P = 0.40). In this study, 25% of the volunteers were black (African Americans), and subjects were recruited based on CYP3A5 genotype (Kharasch et al., 2007). The overall effect was constant if this study was excluded [WMD 0.18 (95% CI 0.09–0.26) l/h/kg; P < 0.001], which demonstrated the reliability of the result.

### MDZ Systemic Clearance

Seven studies including 309 healthy volunteers were included for analysis (Greenblatt et al., 1984; Thummel et al., 1996; Gorski et al., 1998, 1999, 2003; Chen et al., 2006b; Kharasch et al., 2007). There was significant heterogeneity (I² = 72%; P = 0.002) among these studies, and they was combined using a random-effects model. Pooling data from these studies showed significantly higher MDZ systemic clearance in women than in men [WMD 0.08 (95% CI 0.03–0.13) l/h/kg; P = 0.002] (Fig. 2). There was no change in the result when a fixed-effects model was used [WMD 0.08 (95% CI 0.05–0.10) l/h/kg; P < 0.001]. This observed heterogeneity was greatly reduced after exclusion of the studies by Kharasch et al. (2007) and Gorski et al. (1999) (I² = 40%; P = 0.16). It should be pointed out that the study by Gorski et al. (1999) was conducted on elderly volunteers (published as an abstract), and the subjects in the study by Kharasch et al. (2007) was chosen based on CYP3A genotype. Exclusion of both studies from the meta-analysis did not cause an obvious change in the result [WMD 0.05 (95% CI 0.02–0.08) l/h/kg; P = 0.001]. No significant difference in
the result was seen if we analyzed the studies using intravenous bolus and intravenous infusion separately (data not shown).

**Extent of Sex-Dependent Differences in Terms of MDZ Oral and Systemic Clearance.** In the current meta-analysis, women exhibited 0.08 l/h/kg higher MDZ systemic clearance and 0.2 l/h/kg higher oral clearance than men (P < 0.001). MDZ oral clearance was approximately 3-fold higher than systemic clearance (mean CL<sub>po</sub> = 1.26 l/h/kg and mean CL<sub>iv</sub> = 0.40 l/h/kg, weighted by the sample size of each study). The extent of the sex-dependent difference in MDZ oral clearance was comparable to that of MDZ systemic clearance (16 versus 20%). However, the studies included in the meta-analysis of MDZ oral clearance were not necessarily included in the analysis of MDZ systemic clearance and vice versa. There were six studies (Thummel et al., 1996; Gorski et al., 1998, 1999, 2003; Chen et al., 2006b; Kharasch et al., 2007) in common for the analysis of MDZ oral and systemic clearance. We did a further meta-analysis of these studies that had both oral and systemic clearance to demonstrate the reliability of the above-mentioned result. We still found that women exhibited significantly higher MDZ oral clearance [WMD 0.29 (95% CI 0.01–0.56) l/h/kg; P = 0.04] and systemic clearance [WMD 0.08 (95% CI 0.02–0.13) l/h/kg; P = 0.008] than men. With respect to the comparison of the extent of the sex-dependent differences, no obvious change in the result was seen (23 versus 20%).

**AUC of Oral MDZ.** Eight studies including an unpublished study (Z.-Y. Hu, Q. Yu, and Z.-N. Cheng) with a total of 307 healthy volunteers provided AUC in women and men (Thummel et al., 1996; Gorski et al., 1998, 2003; Chen et al., 2006b; Kharasch et al., 2007; Quinney et al., 2008; Hu et al., 2009). MDZ oral dosage varied among these studies, and the magnitude of AUC values depended largely on the dose given. As a result, we calculated SMD instead of WMD. Statistical heterogeneity was not detected among these studies (I<sup>2</sup> = 0.40), so a fixed-effects model was used. Meta-analysis showed no detectable difference in AUC<sub>po</sub> between women and men [SMD −0.05 (95% CI −0.28–0.17); P = 0.64] (Fig. 2).

**AUC of Intravenous MDZ.** A meta-analysis of pooled data from six studies (Thummel et al., 1996; Gorski et al., 1998, 2003; Chen et al., 2006b; Kharasch et al., 2007; Quinney et al., 2008) with 269 healthy volunteers showed that AUC<sub>iv</sub> was lower in women than in men [SMD −0.29 (95% CI −0.53 to −0.04); P = 0.02] (Fig. 2). Heterogeneity was not significant between these studies (I<sup>2</sup> = 40%, P = 0.14), so a fixed-effects model was used.

**MDZ Oral Bioavailability.** A meta-analysis of pooled data from six studies (Greenblatt et al., 1984; Thummel et al., 1996; Gorski et al., 1998, 2003; Kharasch et al., 2007; Quinney et al., 2008) showed no detectable difference in MDZ F between women and men [WMD −0.01 (95% CI −0.03–0.02) l/h/kg, P = 0.63; heterogeneity I<sup>2</sup> = 37%, P = 0.16] (Fig. 3).

**MDZ Gastrointestinal Extraction.** A meta-analysis of pooled data from five studies (Thummel et al., 1996; Gorski et al., 1998, 2003; Kharasch et al., 2007; Quinney et al., 2008) showed no significant difference in MDZ E<sub>gi</sub> between sexes [WMD −0.06 (95% CI −0.15–0.04) l/h/kg, P = 0.25; heterogeneity I<sup>2</sup> = 57%, P = 0.05] (Fig. 3).

**Plasma Ratio of OM versus MDZ.** A published study showed that the value of log OM/MDZ was significantly higher in women than in men (median −0.36 versus −0.43, P < 0.05; 104 women and 98 men) (Zhu et al., 2003). Although a suboptimal index for CYP3A activity was used, the result of this study could serve as indirect evidence to support the conclusion that women exhibit higher hepatic or intestinal CYP3A activity than men.

**Discussion**

This study has several potential limitations. First, the possibility of publication and selection biases cannot be completely excluded because we were unable to obtain data from three eligible studies. To assess the influence of these three studies on our results, we attempted to include these studies in our meta-analysis with the assumed data. None of the three studies found a significant sex difference in MDZ clearance, and only combined MDZ clearance data were provided. Hence, we assumed the MDZ clearance to be the same in women and men. If weight-corrected MDZ clearance was not available, we calculated it based on the assumed weight of women and men. Inclusion of these studies did not change the result of our meta-analysis. The extent of sex-dependent differences in oral and systemic clearance was 14 and 17%, respectively. In the present meta-analysis, there was no evidence of publication bias from either the funnel plot or the Egger test. As a caution, we also calculated the “fail-safe N” to assess the potential for publication bias to have influenced the results of our meta-analysis. Fail-safe N can be defined as the number of new, unpublished, or unretrieved nonsignificant studies that would be required to exist to lower the significance of a meta-analysis to nonsignificant (Rosenthal, 1979; Rosenberg, 2005). The fail-safe N was 58 and 89 (0.05 significance level) for sex difference in MDZ oral clearance and systemic clearance, respectively. The existence of that many unpublished studies is improbable, so the results of our meta-analysis were robust. Second, there was significant heterogeneity among the studies analyzed, probably caused by differences in study population, race/ethnicity, dose administered, dosing regimen, and dosage formulation. It should also be pointed out that detection of sex differences was not the major aim in several included studies. Third, retrospective mean data of 13 independent clinical studies were in-
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women exhibited 11% higher mean weight-corrected MDZ systemic clearance and 28% higher oral clearance than men (P ≤ 0.01) (Chen et al., 2006b).

A retrospective analysis of the 13 clinical studies showed that

inclided in the meta-analysis because of the difficulties in obtaining raw data for each of the clinical studies (Chen et al., 2006b).

FIG. 2. Forest plots for sex-dependent differences in CLpo (liters per hour per kilogram), CLiv (liters per hour per kilogram), AUCpo (micrograms per hour per liter), and AUCiv (micrograms per hour per liter). WMD values or SMD values are shown with 95% confidence intervals.

Heterogeneity: Chi² = 8.33, df = 5 (P = 0.14); I² = 40%

Test for overall effect: Z = 0.47 (P = 0.64)

Heterogeneity: Tau² = 0.00; Chi² = 21.32, df = 6 (P = 0.002); I² = 72%

Test for overall effect: Z = 3.13 (P = 0.002)

Heterogeneity: Chi² = 11.89, df = 7 (P = 0.10); I² = 41%

Test for overall effect: Z = 4.74 (P < 0.00001)

Heterogeneity: Chi² = 7.33, df = 7 (P = 0.40); I² = 4%

Test for overall effect: Z = 0.47 (P = 0.64)
studies (41 women and 58 men) showed that systemic clearances were 30% greater in women, but there was no sex difference for oral MDZ (Kharasch et al., 2007). Both vivo studies showed that the extent of sex-dependent differences in MDZ oral clearance was much different from that in systemic clearance, suggesting that sex differences in MDZ F may exist. However, a meta-analysis of pooled data from six studies (Greenblatt et al., 1984; Thummel et al., 1996; Gorski et al., 1998, 2003; Kharasch et al., 2007; Quinney et al., 2008) showed no sex difference in either MDZ CYP3A4 hepatic content, and CYP3A4 mRNA have been reported in white women (CYP3A4). This conclusion was supported by the in vitro studies conducted on a large number of samples. For example, Thummel et al. (1996) showed that women have higher hepatic CYP3A activity than men, whereas no sex-dependent difference existed in intestine. This conclusion was supported by the in vitro studies conducted on a large number of samples. For example, higher hepatocyte CYP3A44 activity (Parkinson et al., 2004), higher CYP3A44 hepatic content, and CYP3A4 mRNA have been reported in white women (n = 46) compared with white men (n = 48) (Wobold et al., 2003). In contrast, Paine et al. (2005) found a lack of a sex-dependent difference in expression levels of intestinal CYP3A4 (45 women and 46 men).

In the present study, no sex-dependent difference was noted in MDZ AUCpo (P = 0.85). However, AUCvs lower in women than in men (P = 0.02). The observed difference, although statistically significant, was moderate. This suggests that sex differences in MDZ exposure may not be important.

If we stratified the included studies into subgroups representing different races, no ethnic difference was observed. For example, pooled data from Asians showed significantly higher MDZ oral clearance in women than in men [WMD 0.22 (95% CI 0.08–0.37) l/h/kg; P = 0.003]. Similar results were observed in whites and other races [WMD 0.18 (95% CI 0.09–0.28) l/h/kg; P < 0.001]. This result was reasonable because the pharmacokinetics of MDZ is similar in white subjects and East Asians after weight correction (Tateishi et al., 2001).

To assure that only the studies with good-quality data were included in our study, we excluded one retrospective study (Miao et al., 2009) and four clinical studies with small sample sizes (Lown et al., 1995; Tsunoda et al., 1999; He et al., 2005; Rocha et al., 2008). Excluded in our study, we excluded one retrospective study (Miao et al., 2009) and four clinical studies with small sample sizes (Lown et al., 1995; Tsunoda et al., 1999; He et al., 2005; Rocha et al., 2008). However, inclusion of these studies in our study will not change the general result of our study (data not shown).

The clinical implication of this meta-analysis must be considered. In clinical therapeutics, doses of most medications metabolized by CYP3A are not adjusted based on body size or sex, with women and men receiving the same medication doses per clinical guidelines for the condition. If a fixed absolute dose were administered to men and women, the 20% higher weight-normalized clearance in women would be offset by their lower average body weight. Under this circumstance, the exposure of medications was expected to be similar in women and men. However, in clinical practice, the doses of some medications are adjusted based on body size (e.g., alfentanil). Considering the lower average body weight of women, women are often given lower doses of such medications than men. Therefore, women may be underdosed, suggesting that the dosing regimen should be adjusted accordingly (e.g., increase doses or shorten dosing interval).

The goal of clinical pharmacology is to assure that the right dose of the right drug is administered to the patient. Accomplishing this goal will require that sex-specific medication-prescribing information be incorporated into clinical therapeutics.

Considering the conclusive finding of this study, we believe that there may be no need for further studies to test whether there is a sex
difference in CYP3A activity. However, there is a need for studies that focus on the underlying mechanism of sex difference in CYP3A and also on the effect of this sex difference on clinical outcome.

In conclusion, women showed significantly greater hepatic CYP3A activity than men, whereas no sex-dependent difference in intestinal CYP3A activity was observed. To our knowledge, this sex-based study is the most comprehensive analysis that has examined CYP3A activity by use of MDZ as a probe. Although a sex-dependent difference in CYP3A activity was statistically significant, this may or may not translate into differences in pharmacodynamics or clinical outcome (Chen, 2005).

Acknowledgments. We kindly acknowledge Wei Jia for screening relevant articles from reference lists and Dai-Li Xi for valuable comments and suggestions regarding this article.

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