Increased Glyburide Clearance in the Pregnant Mouse Model

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ABSTRACT:
Glyburide (GLB) is an oral sulfonylurea, commonly used for the treatment of gestational diabetes mellitus. It has been reported that the clearance of GLB in pregnant women is significantly higher than that in nonpregnant women. The molecular mechanism by which pregnancy increases the clearance of GLB is not known, but it may be caused by increased CYP3A activity. Because liver tissue from pregnant women is not readily available, in the present study, we investigated the mechanism of such pregnancy-related changes in GLB disposition in a mouse model. We demonstrated that the systemic clearance of GLB in pregnant mice was increased approximately 2-fold ($p < 0.01$) compared with nonpregnant mice, a magnitude of change similar to that observed in the clinical study. Plasma protein binding of GLB in mice was not altered by pregnancy. The half-life of GLB depletions in hepatic S-9 fractions of pregnant mice was significantly shorter than that of nonpregnant mice. Moreover, GLB depletion was markedly inhibited by ketoconazole, a potent inhibitor of mouse Cyp3a, suggesting that GLB metabolism in mice is primarily mediated by hepatic Cyp3a. These data suggest that the increased systemic clearance of GLB in pregnant mice is likely caused by an increase in hepatic Cyp3a activity during pregnancy, and they provide a basis for further mechanistic understanding and analysis of pregnancy-induced alterations in the disposition of GLB and drugs that are predominantly and extensively metabolized by CYP3A/Cyp3a.

Introduction
Glyburide (GLB), an oral sulfonylurea, hypoglycemic agent, has been used for the treatment of gestational diabetes mellitus because of its limited placental transfer, similar efficacy to insulin, and ease of administration (Langer et al., 2000). Our recent clinical study demonstrated that the apparent oral clearance of GLB in pregnant women with gestational diabetes mellitus was increased approximately 2-fold compared with that in the control nonpregnant women with type II diabetes mellitus (Hebert et al., 2009). The molecular mechanism by which pregnancy increases the oral clearance of GLB is not known. An increase in oral clearance of a drug (CL/F) could be attributable to an increase in hepatic and/or renal clearance (CL) or a decrease in oral bioavailability (F). GLB is extensively metabolized by the liver. There is no significant renal clearance of the drug (Hebert et al., 2009). GLB is well absorbed with an oral bioavailability of approximately 95% (Jonsson et al., 1994), indicating that the first-pass effect of GLB is likely to be minor. Consequently, we have hypothesized that the significant increase in apparent oral clearance of GLB during pregnancy is likely caused by an increase in hepatic clearance of the drug. This hypothesis is supported by the finding that the formation clearance of the major GLB metabolite, M1, in pregnant patients was increased by 130% compared with that in nonpregnant controls (Hebert et al., 2009).

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ABBREVIATIONS: GLB, glyburide; CL, clearance; F, oral bioavailability; ER, extraction ratio; BCRP, breast cancer resistance protein; fu, fraction unbound; KTZ, ketoconazole; AUC, area under plasma concentration-time curve; MRT, mean residence time; $V_{ss}$, steady-state volume of distribution; $T_{1/2}$, terminal half-life; P450, cytochrome P450.

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mice. We then determined whether the intrinsic clearance of GLB in the liver of pregnant mice was increased by measuring GLB depletion by hepatic S-9 fractions and if the increase in GLB depletion was caused by increased hepatic Cyp3a activity. We have previously shown that GLB is a substrate of human breast cancer resistance protein (BCRP) and its murine homolog Bcrp1 (Zhou et al., 2008). Because Bcrp1 expression in the liver and kidney is induced by pregnancy at midgestation (Wang et al., 2006), we also performed similar pharmacokinetic studies in Bcrp1(−/−) mice to assess the role of Bcrp1 in GLB disposition.

Materials and Methods

Animal Studies. All of the materials and animals, which included GLB, [3H]GLB, polyethylene glycol 400, FVB wild-type mice, and Bcrp1(−/−) mice, were the same as those described previously (Zhou et al., 2008). The animal study protocol was approved by the Institutional Animal Care and Use Committee of the University of Washington. Feeding and maintenance of mice, weight and age of mice, mating, estimation of gestational age, and monitoring progression of pregnancy were essentially the same as those described previously (Zhou et al., 2008). Pregnant mice used were at day 15 of gestation. GLB was dissolved in a solvent [0.5% (v/v) dimethyl sulphoxide, 10% (v/v) ethanol, 39.5% (v/v) saline, and 50% (v/v) polyethylene glycol 400] at 0.5 mg/ml. Under anesthesia (isoflurane), GLB (1 mg/kg b.wt.) was administered to pregnant or nonpregnant mice by retro-orbital injection. At 0.5, 5, 10, 20, 30, 40, 60, 120, 180, and 240 min after drug administration, 3 to 5 mice per time point were sacrificed under anesthesia by cardiac puncture. Immediately thereafter, liver tissues were harvested and stored at −80°C until use. Blood was collected in heparinized microcentrifuge tubes (BD Biosciences, Franklin Lakes, NJ) and centrifuged at 1,500g at room temperature for 10 min. The harvested plasma samples were stored at −20°C until analysis. GLB concentrations in mouse plasma samples were determined using a validated high-performance liquid chromatography/mass spectrometry assay as described previously (Zhou et al., 2008).

Plasma Protein Binding. Mouse plasma protein binding of GLB was determined by ultrafiltration using Millipore Centrifree ultrafiltration (Millipore Corporation, Billerica, MA) cartridges as described previously (Hebert et al., 2009). In brief, [3H]GLB (40 ng) in methanol was aliquoted into disposable culture tubes and evaporated to dryness. One milliliter of GLB-free blank plasma from pregnant or nonpregnant mice spiked with nonradioactive GLB was added to each tube. The samples were then mixed well and allowed to equilibrate at 37°C for at least 30 min. Three aliquots (0.3 ml each) of the samples from each tube were transferred to ultrafiltration cartridges, equilibrated at 37°C for 30 min, and centrifuged at 1,000g for 15 min at 37°C. Thirty microliters of the filtrates and unfiltered plasma were counted on a liquid scintillation counter. The fraction unbound (fu) of GLB was calculated as the percentage of the radioactivity of the filtrates to the radioactivity of the plasma from pregnant or nonpregnant mice by retro-orbital injection. At 0.5, 5, 10, 20, 30, 40, 60, 120, 180, and 240 min after drug administration, 3 to 5 mice per time point were sacrificed under anesthesia by cardiac puncture. Immediately thereafter, liver tissues were harvested and stored at −80°C until use. Blood was collected in heparinized microcentrifuge tubes (BD Biosciences, Franklin Lakes, NJ) and centrifuged at 1,500g at room temperature for 10 min. The harvested plasma samples were stored at −20°C until analysis. GLB concentrations in mouse plasma samples were determined using a validated high-performance liquid chromatography/mass spectrometry assay as described previously (Zhou et al., 2008).

GLB Depletion. Mouse hepatic S-9 fractions were prepared as described previously (Mathias et al., 2006). GLB depletion reaction mixtures contained 100 mM phosphate-buffered saline (pH 7.4), 1 mg/ml S-9 fractions, 5 mM MgCl2, and 0.16 to 1.25 μM GLB dissolved in 1% (v/v) acetonitrile, in a final volume of 200 μl, in the absence or presence of 1 μM ketoconazole (KTZ). After predworking for 5 min, reactions were initiated by adding the NADPH-regenerating system (1 mM NADP+, 10 mM glucose 6-phosphate, and 1 unit/ml glucose-6-phosphate dehydrogenase). Incubations with the regenerating system or 1% acetonitrile alone were added as negative controls. Reactions were stopped at 0, 5, 10, or 20 min by adding 2 ml of the mixed solvent (n-hexane/methylene chloride at a 1:1 ratio, v/v). Each sample was then acidified by adding 20 μl of HCl (2 M), and 20 μl of glipizide (2 μM) (internal standard) dissolved in acetonitrile was added. The samples were briefly vortexed at room temperature, and the upper organic phase was transferred to a disposable clean glass tube and dried under N2. The dried residue was reconstituted in 100 μl of a mixed solvent (methanol/ H2O at a 20:80 ratio with 0.5 mM ammonium formate). Fifteen microliters of each reconstituted sample were injected, and the GLB concentrations were determined using a validated high-performance liquid chromatography/mass spectrometry assay as described previously (Zhou et al., 2008). Half-life of GLB depletion was calculated according to the first-order decay kinetics by linear regression.

Pharmacokinetic Data Analysis. Due to the nature of the data (one blood sample from each mouse), the Bailey’s approach (Bailer, 1988) was used to estimate the mean and S.E. of the maternal plasma areas under plasma concentration-time curve (AUCs) and other pharmacokinetic parameters including the mean residence time (MRT), CL, and steady-state volume of distribution (Vss), and the normal hypothesis test was performed to assess the statistically significant difference of each parameter between two animal groups, as described previously (Zhou et al., 2008).

GLB was administered to pregnant or nonpregnant mice based on body weight. Because the body weight of a pregnant mouse is usually 1.5 times greater than that of a nonpregnant mouse, pregnant mice received a larger dose. Body weights (mean ± S.D.) of nonpregnant wild-type, pregnant wild-type, nonpregnant Bcrp1(−/−), and pregnant Bcrp1(−/−) mice were 22.9 ± 1.7, 31.2 ± 2.3, 21.2 ± 2.2, and 28.9 ± 3.6 g, respectively. We have previously shown that GLB in the fetuses of pregnant mice only accounts for a small fraction of the total amount of GLB in the body (Zhou et al., 2008), suggesting that the fetus is not a major site for GLB distribution. Hence, we estimated dose-normalized AUC and total plasma CL of GLB as follows.

- CL = (mean actual dose of the respective mouse group)/AUC0.5–240 min
- AUCdose-normalized = AUC0.5–240 min/(mean actual dose of the respective mouse group)

Statistical Analysis. Except for pharmacokinetic parameters that were reported as mean ± S.E., all other data were presented as means ± S.D. Differences between the two animal groups, pregnant mice versus nonpregnant mice, were analyzed by the normal hypothesis test or the Student’s t test. Differences with p values <0.05 were considered statistically significant.

Results and Discussion

After intravenous administration, the dose-normalized maternal plasma concentrations of GLB in FVB wild-type pregnant mice tended to be lower than those in FVB wild-type nonpregnant mice.
As a result, the dose-normalized AUC of GLB was increased approximately 2-fold by pregnancy. Our data suggest that the intrinsic activity of GLB metabolism by hepatic S-9 fractions is significantly increased by pregnancy. Because the total protein yield in S-9 fractions normalized to body weight was not significantly affected by pregnancy, in preliminary depletion experiments, we determined the optimal protein concentration of S-9 fractions to be 1 mg/ml, which allows GLB depletion to follow the first-order decay kinetics. In addition, we found that incubation times of up to 20 min were optimal so that a sufficient amount of GLB was depleted for an accurate estimation of metabolic activity, and, at the same time, there was enough GLB remaining for accurate quantification of the drug. The half-life of GLB depletion was found to be unchanged over concentrations ranging from 0.16 to 1.25 μM. Therefore, all subsequent GLB depletion experiments were carried out with 0.625 μM GLB and 1 mg/ml S-9 fractions for incubation of up to 20 min. The half-life of GLB depletion in S-9 fractions of wild-type pregnant mice (13.0 ± 1.4 min) was 37% lower (p < 0.05) than that of wild-type nonpregnant mice (20.6 ± 4.6 min) (Fig. 2A). This result suggests that the intrinsic activity of GLB metabolism by hepatic S-9 fractions is significantly increased by pregnancy. Because the total protein yield in S-9 fractions was not significantly changed by pregnancy after normalization to body weight, we believe that the systemic clearance of GLB in pregnancy is increased by an induction in intrinsic clearance of GLB in the liver, rather than an increase in the amount of hepatic proteins in pregnant mice.

To determine whether GLB depletion by hepatic S-9 fractions is catalyzed by Cyp3a, we determined the effect of Cyp3a inhibition on GLB depletion. After incubation for 20 min, there was greater depletion of GLB in hepatic S-9 fractions of wild-type pregnant mice (~70%) compared with wild-type nonpregnant mice (~50%) (Fig. 2B). However, this depletion was significantly inhibited by 1 μM KTZ (Fig. 2B). Because KTZ is a potent inhibitor of mouse Cyp3a (Mathias et al., 2006), these data suggest that hepatic GLB depletion in mice is primarily mediated by Cyp3a, and the increased GLB depletion by hepatic S-9 fractions of pregnant mice is likely caused by an increase in intrinsic activity of hepatic Cyp3a.

We have previously shown that the activity of human CYP3A is elevated in vivo during pregnancy (Hebert et al., 2008). The activity of mouse hepatic Cyp3a, determined using testosterone 6β-hydroxylation as a marker activity, is similarly induced by pregnancy (Mathias et al., 2006; Zhang et al., 2008). Therefore, we postulate that the increase in systemic clearance of GLB in pregnant mice is likely caused by the increased intrinsic activity of hepatic Cyp3a during pregnancy. Approximately 15% of GLB was still depleted even in the presence of 1 μM KTZ, which is presumably sufficient to fully inhibit

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT Pregnant</th>
<th>WT Nonpregnant</th>
<th>p Value</th>
<th>Bcrp1(−/−) Pregnant</th>
<th>Bcrp1(−/−) Nonpregnant</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC&lt;sub&gt;dose-normalized&lt;/sub&gt; [μg · min/ml]/μg</td>
<td>1.8 ± 0.1</td>
<td>3.2 ± 0.1</td>
<td>&lt;0.01</td>
<td>1.7 ± 0.1</td>
<td>3.3 ± 0.1</td>
<td>&lt;0.01</td>
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<tr>
<td>CL (ml/min)</td>
<td>0.56 ± 0.03</td>
<td>0.31 ± 0.00</td>
<td>&lt;0.01</td>
<td>0.60 ± 0.03</td>
<td>0.31 ± 0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>63.2 ± 7.7</td>
<td>49.5 ± 3.3</td>
<td>&gt;0.05</td>
<td>56.7 ± 4.6</td>
<td>51.0 ± 3.9</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (min)</td>
<td>43.8 ± 8.8</td>
<td>34.3 ± 4.1</td>
<td>&gt;0.05</td>
<td>39.3 ± 6.0</td>
<td>35.4 ± 4.7</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>V&lt;sub&gt;ss&lt;/sub&gt; (ml)</td>
<td>35.3 ± 4.6</td>
<td>15.6 ± 1.2</td>
<td>&lt;0.05</td>
<td>34.2 ± 3.2</td>
<td>15.6 ± 1.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>fu (%)</td>
<td>3.4 ± 0.3</td>
<td>3.2 ± 0.3</td>
<td>&gt;0.05</td>
<td>3.9 ± 0.4</td>
<td>3.6 ± 0.3</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

**TABLE 1**

Pharmacokinetic parameters of glyburide in pregnant and nonpregnant FVB wild-type (WT) or Bcrp1(−/−) mice after retro-orbital administration at a dose of 1 mg/kg b.w.t.
On the other hand, if such medications are titrated to response during adjustment may be required to maintain efficacy during pregnancy. Substrates with a narrow therapeutic index may fall below their (Lilja et al., 2007). Plasma concentrations of drugs that are CYP3A inducers or inhibitors are to be coadministered with GLB to metabolism (Naritomi et al., 2004; Zharikova et al., 2009; Zhou et al., induction of hepatic Cyp3a activity by pregnancy. Given that CYP3A4, human CYP3A5, CYP2C19, CYP2C8, and CYP2C9 are also capable of metabolizing GLB (Zhou et al., 2010). We also observed that pregnancy significantly increased Vss of GLB (Table 1) possibly due to the increase in both total body water and fat content during pregnancy, which suggests that distribution of GLB into maternal tissues is likely increased in pregnancy. Because the CL of GLB was also increased, this observation is consistent with the fact that T1/2 of GLB (T1/2 = 0.693 \cdot Vss/CL) was not significantly altered by pregnancy (Table 1). In a clinical setting with multiple GLB oral dosing, this change in Vss would not be expected to affect the average steady-state plasma concentration of GLB, because Cavg of GLB is determined by its dosing rate and clearance [Cavg = (dose/interval)/oral CL].

In summary, in the present study, we have illustrated that the increased systemic clearance of GLB in pregnant mice is likely due to induction of hepatic Cyp3a activity by pregnancy. Given that CYP3A4 is the major human P450 enzyme responsible for GLB metabolism (Naritomi et al., 2004; Zharikova et al., 2009; Zhou et al., 2010), these data support the hypothesis that the pregnancy-induced increase in the clearance of GLB in humans is also primarily caused by an increase in hepatic CYP3A activity. Such findings have significant clinical implications. For example, caution should be taken when CYP3A inducers or inhibitors are to be coadministered with GLB to avoid potential adverse drug-drug interactions in pregnant women (Lilja et al., 2007). Plasma concentrations of drugs that are CYP3A substrates with a narrow therapeutic index may fall below their effective therapeutic concentrations in pregnancy, and therefore dose adjustment may be required to maintain efficacy during pregnancy. On the other hand, if such medications are titrated to response during pregnancy, a dose reduction may be needed postpartum to avoid potential toxicity. Our data suggest that the FVB mouse may be an appropriate animal model to study the effect of pregnancy on the disposition of drugs that are predominantly and extensively metabolized by hepatic CYP3A/Cyp3a and to investigate the molecular mechanism by which pregnancy induces CYP3A/Cyp3a activity.

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References


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