Increased Glyburide Clearance in the Pregnant Mouse Model

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d) Abbreviations:

CYP, cytochrome P450; GLB, glyburide; AUC, the area under plasma concentration-time curve; CL, clearance; F, oral bioavailability; V_{ss}, steady-state volume of distribution; MRT, mean residence time; T_{1/2}, terminal half-life; ER, extraction ratio; fu, fraction unbound; PEG 400, polyethylene glycol 400; PBS, phosphate buffered saline; KTZ, ketoconazole; BCRP, breast cancer resistance protein; OATP2B1, organic anion-transporting polypeptide 2B1.
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 non-pregnant women. The molecular mechanism by which pregnancy increases the clearance of GLB is not known, but may be caused by increased CYP3A activity. As 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) as compared with non-pregnant 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 depletion in hepatic S-9 fractions of pregnant mice was significantly shorter than that of non-pregnant 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 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 as with 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 when compared with that in the control non-pregnant 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 not a 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% as compared with that in non-pregnant controls (Hebert et al., 2009).

GLB is a low hepatic extraction ratio (ER) drug (ER ~0.1) in humans (Jonsson et al., 1994). For a low ER drug, based on the well-stirred model, hepatic clearance of the drug is approximated by the product of fraction unbound in blood and intrinsic clearance of the drug in the liver (Rowland and Tozer, 1995). Since there is no observed change in plasma protein binding for GLB during pregnancy (Hebert et al., 2009), an increase in hepatic clearance of GLB
can be explained by an increase in intrinsic clearance of GLB in the liver. Increased intrinsic clearance of GLB in the liver is likely caused by an increase in the activity of the major metabolizing enzyme of GLB. Our recent in vitro metabolism studies using human liver microsomes and the studies reported by others have revealed that CYP3A plays a major role in in vitro metabolism of GLB (Naritomi et al., 2004; Zharikova et al., 2009; Zhou et al., 2010). Hence, we further hypothesize that pregnancy induces the activity of hepatic CYP3A, resulting in an increase in oral clearance of GLB. To test this hypothesis, an animal model would be required, as liver tissue from pregnant women is not readily available. The mouse model was used in our study for two reasons. First, we have shown that both the protein levels of hepatic Cyp3a in pregnant mice and its activity measured using testosterone as the probe substrate are significantly increased as compared with those in non-pregnant controls (Mathias et al., 2006; Zhang et al., 2008). Second, the activity of hepatic CYP3A in humans has also been shown to be induced by pregnancy, based on the observation of increased metabolism of probe drugs predominantly catalyzed by CYP3A such as midazolam (Hebert et al., 2008). This induction appears to be due to increased expression of the CYP3A/Cyp3a gene possibly mediated by pregnancy-related hormones or growth factors (Zhang et al., 2008).

Therefore, in the present study, we first investigated whether pregnancy increases the systemic clearance of GLB in wild-type FVB 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 BCRP and its murine homolog Bcrp1 (Zhou et al., 2008). Since Bcrp1 expression in the liver and kidney is
induced by pregnancy at mid-gestation (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 the materials and animals including GLB, [3H]-GLB, polyethylene glycol 400 (PEG 400), and FVB wild-type and Bcrp1−/− mice were the same as previously described (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 previously described (Zhou et al., 2008). Pregnant mice used were at day 15 of gestation. GLB was dissolved in a solvent (0.5% (v/v) dimethyl sulfoxide, 10% (v/v) ethanol, 39.5% (v/v) saline and 50% (v/v) PEG 400) at 0.5 mg/ml. Under anesthesia (isoflurane), GLB (1 mg/kg body weight) was administered to pregnant or non-pregnant mice by retro-orbital injection. At times 0.5, 5, 10, 20, 30, 40, 60, 120, 180, and 240 min after drug administration, 3 – 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 (Becton Dickinson, Franklin Lakes, NJ) and centrifuged at 1,500 × g 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 HPLC/MS assay as previously described (Zhou et al., 2008).

Plasma protein binding. Mouse plasma protein binding of GLB was determined by ultrafiltration using Millipore Centrifree ultrafiltration cartridges as described (Hebert et al., 2009). Briefly, [3H]-GLB (40 ng) in methanol was aliquoted into disposable culture tubes and evaporated to dryness. One ml of GLB-free blank plasma from pregnant or non-pregnant mice
spiked with cold 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 1,000 × g 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 corresponding unfiltered plasma. Preliminary experiments showed constant GLB protein binding over the range of 138 – 6,040 ng/ml. Non-specific binding of [³H]-GLB was determined by filtering 0.3 ml of phosphate buffered saline (PBS) containing 40 ng/ml [³H]-GLB. Non-specific binding of [³H]-GLB was around 20%.

GLB depletion. Mouse hepatic S-9 fractions were prepared as previously described (Mathias et al., 2006). GLB depletion reaction mixtures contained 100 mM PBS (pH 7.4), 1 mg/ml of 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. After a pre-warming 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 used 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 2 M HCl, and 20 µl of 2 µM glipizide (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 HPLC/MS assay as previously described (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 Bailer’s approach (Bailer, 1988) was used to estimate the mean and standard error (S.E.) of the maternal plasma AUCs and other pharmacokinetic parameters including the mean residence time (MRT), clearance (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 previously described (Zhou et al., 2008).

GLB was administered to pregnant or non-pregnant mice based on body weight. Since the body weight of a pregnant mouse is usually 1.5 times greater than that of a non-pregnant mouse, pregnant mice received a larger dose. Body weights (mean ± S.D.) of non-pregnant wild-type, pregnant wild-type, non-pregnant 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 clearance (CL) of GLB as follows.

\[
CL = \frac{\text{mean actual dose of the respective mouse group}}{\text{AUC}_{0.5-240 \text{ min}}} \\
\text{AUC}_{\text{dose-normalized}} = \frac{\text{AUC}_{0.5-240 \text{ min}}}{\text{mean actual dose of the respective mouse group}}
\]
Statistical analysis. Except for pharmacokinetic parameters which were reported as means ± S.E., all other data were presented as means ± S.D. Differences between the two animal groups, pregnant mice vs. non-pregnant 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 non-pregnant mice (Figure 1A). As a result, the dose-normalized AUC (AUC_dose-normalized) in wild-type pregnant mice was 44% lower (p < 0.01) compared with that in wild-type non-pregnant mice (Table 1). Consequently, the clearance of GLB (CL) was increased approximately 2-fold by pregnancy. The steady-state volume of distribution (Vss) of GLB was 126% higher (p < 0.05) during pregnancy. MRT, T1/2, and fraction unbound (fu) of GLB in plasma in wild-type pregnant and non-pregnant mice were not significantly different (Table 1). The magnitude of changes in dose-normalized AUC and CL were similar to those observed in humans (Hebert et al., 2009). Since GLB was administered intravenously, the effect of pregnancy on the systemic clearance likely reflects change in hepatic metabolism of GLB. For GLB, a low ER drug with no significant renal clearance, the increase in systemic clearance would be mainly caused by an increase in hepatic clearance, which is determined by the fraction unbound in plasma and the intrinsic clearance of GLB in the liver. Our data indicate that the increase in systemic clearance of GLB in pregnant mice is unlikely caused by change in fraction unbound because there is no observed alteration in mouse plasma protein binding by pregnancy. Likewise, plasma protein binding of GLB in humans was not altered by pregnancy (Hebert et al., 2009).

Similar results were obtained in Bcrp1⁻/⁻ mice as in wild-type mice, no matter whether the mice were pregnant or non-pregnant (Figure 1B and Table 1). Therefore, Bcrp1 appears to play only a minor role in the systemic clearance of GLB in mice. Consequently, the role of Bcrp1 in the systemic clearance of GLB was not further investigated. GLB is a substrate of other
transporters such as P-glycoprotein (Golstein et al., 1999) and organic anion-transporting polypeptide OATP2B1 (Satoh et al., 2005). Whether these transporters play a role in the systemic clearance of GLB is not known. However, since the protein levels of P-glycoprotein in the mouse liver or kidney are not significantly altered by pregnancy (Zhang et al., 2008), we expect that P-glycoprotein-mediated elimination of GLB is likely not to be significantly changed by pregnancy.

We next examined whether the increase in systemic clearance of GLB during pregnancy is possibly caused by an increase in hepatic depletion of the drug. We analyzed GLB depletion by S-9 fractions isolated from liver tissues of pregnant and non-pregnant wild-type mice. We first measured the yield of total hepatic protein recovered in the S-9 fractions. The protein contents in hepatic S-9 fractions normalized to liver weight were 285.4 ± 47.0 (mg/g liver) and 209.8 ± 3.1 (mg/g liver) for non-pregnant and pregnant mice, respectively. The liver weights per mouse for non-pregnant and pregnant mice were 0.64 ± 0.07 (g/mouse) and 1.11 ± 0.09 (g/mouse), respectively. The liver weights of non-pregnant and pregnant mice normalized to body weight were 27.8 ± 3.1 (g/kg body weight) and 35.6 ± 2.8 (g/kg body weight), respectively. Therefore, the total hepatic protein contents in the S-9 fractions normalized to body weight were 7844.7 ± 849.2 (mg/kg body weight) and 7479.2 ± 644.4 (mg/kg body weight) for non-pregnant and pregnant mice, respectively. According to these data, there was approximately a 30% increase in total protein amount of hepatic S-9 fractions isolated from pregnant mice compared with non-pregnant mice. This is possibly caused by the increase in liver weight of pregnant mice compared with non-pregnant mice. However, the total protein amount 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 that 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 the concentration range 0.16 to 1.25 μM. Therefore, all subsequent GLB depletion experiments were carried out with 0.625 μM GLB and 1 mg/ml of 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 non-pregnant mice (20.6 ± 4.6 min) (Figure 2A). This suggests that the intrinsic activity of GLB metabolism by hepatic S-9 fractions is significantly increased by pregnancy. Since 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 non-pregnant mice (~50%) (Figure 2B). However, this depletion was significantly inhibited by 1 μM ketoconazole (Figure 2B). Since ketoconazole 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). We therefore 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. Interestingly, approximately 15% of GLB was still depleted even in the presence of 1 μM ketoconazole which is presumably sufficient to fully inhibit Cyp3a (Hickman et al., 1998). This suggests that other mouse hepatic Cyp isoforms and/or non-Cyp enzymes may also contribute to GLB metabolism. We have shown that, in addition to 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, suggesting that distribution of GLB into maternal tissues is likely increased in pregnancy. Since the clearance (CL) of GLB was also increased, this is consistent with the fact that T1/2 of GLB (T1/2 = 0.693 · 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 (Css, ave) of GLB, because Css, ave of GLB is determined by its dosing rate and clearance (Css, ave = (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 CYP 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 co-administered 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


mechanisms, tissue specificity, and time course. *Mol Pharmacol* 74(3):714-723.


Footnotes

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Figure legends

Figure 1. Dose-normalized plasma concentration-time profiles of glyburide in FVB wild-type (A) and Bcrp1⁻/⁻ (B) mice. Pregnant mice at day 15 of gestation and non-pregnant mice were administered GLB (1 mg/kg body weight) by retro-orbital injections. At times 0.5 – 240 min after drug administration, plasma samples were collected. Dose-normalized GLB plasma concentrations were calculated by dividing the GLB plasma concentrations by the mean actual dose of the respective mouse group. Shown are mean ± S.D. (n = 3 – 5 mice per time point).

Figure 2. Time-course of glyburide depletion by mouse hepatic S-9 fractions (A) and inhibition of glyburide depletion by ketoconazole (B). A) GLB depletion was performed by incubating GLB at 0.625 µM with S-9 fractions (1 mg/ml) of wild-type non-pregnant (solid squares, n = 7 per time point) or wild-type pregnant mice (solid triangles, n = 8 per time point) for up to 20 min. The amounts of GLB at time 0 were set as 100%. Shown are mean ± S.D. B) GLB depletion was performed as in A for 20 min in the absence (open bars) or presence (solid bars) of 1 µM ketoconazole. The amounts of GLB in the samples with no NADPH-regenerating system added were set as 100%. Shown are mean ± S.D. of 6 independent determinations. Significant differences: * p < 0.05; ** p < 0.01; *** p < 0.005 by the Student’s t-test.
Table 1. Pharmacokinetic parameters of glyburide in pregnant and non-pregnant FVB wild-type (WT) or Bcrp1<sup>−/−</sup> mice after retro-orbital administration at a dose of 1 mg/kg body weight.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT pregnant</th>
<th>WT non-pregnant</th>
<th>p value</th>
<th>Bcrp1&lt;sup&gt;−/−&lt;/sup&gt; pregnant</th>
<th>Bcrp1&lt;sup&gt;−/−&lt;/sup&gt; non-pregnant</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC dose-normalized [((µg • min/ml)/µg)]</td>
<td>1.8 ± 0.1 3.2</td>
<td>± 0.1 &lt;</td>
<td>0.01</td>
<td>1.7</td>
<td>± 0.1 3.3</td>
<td>± 0.1 &lt;</td>
</tr>
<tr>
<td>CL (ml/min)</td>
<td>0.56 ± 0.03</td>
<td>0.31 ± 0.01</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 ± 1.3</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>fu (%)</td>
<td>3.4 ± 0.3 3.2</td>
<td>± 0.3 &gt;</td>
<td>0.05</td>
<td>3.9</td>
<td>± 0.4 3.6</td>
<td>± 0.3 &gt;</td>
</tr>
</tbody>
</table>

The pharmacokinetic parameters were estimated using the Bailer’s approach (Bailer, 1988). AUC, CL, MRT, T<sub>1/2</sub>, and V<sub>ss</sub> are presented as means ± S.E. (n = 3 – 5 mice per time point), and the fraction unbound fu (%) as mean ± S.D. of 3 – 5 independent determinations. The differences between the pregnant and non-pregnant mice groups shown in this table were analyzed by normal hypothesis test as previously described (Zhou et al., 2008) for the pharmacokinetic data or the Student’s t-test for the unbound fraction data, and differences with p values < 0.05 were considered statistically significant.
Figure 1

A

Dose-normalized GLB plasma concentration ([µg/ml]/µg dose)

- Pregnant FVB wild-type
- Non-pregnant FVB wild-type

Time (min)

B

Dose-normalized GLB plasma concentration ([µg/ml]/µg dose)

- Pregnant Bcrp1\(^{-/-}\)
- Non-pregnant Bcrp1\(^{-/-}\)

Time (min)