Effect of Endotoxin on the Expression of Placental Drug Transporters and Glyburide Disposition in Pregnant Rats

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ABSTRACT:

On average, 80% of pregnant women consume over-the-counter and/or prescription medications. The placenta is a crucial organ that can restrict fetal drug exposure. ATP-binding cassette (ABC) drug transporters play an important role in the placenta because they limit the transplacental transfer of xenobiotics. However, the impact of infection or inflammation on placental drug transporters is not well established. Thus, we examined the impact of endotoxin-induced inflammation on the placental expression of several key drug transporters in rats and its impact on fetal exposure to a drug substrate. Real-time polymerase chain reaction results demonstrated a significant time- and dose-dependent down-regulation of breast cancer resistance protein/Abcg2 mRNA in the placentas of endotoxin-treated rats with a corresponding decrease in protein levels. Likewise, the mRNA levels of several other ABC transporters (Abcb1a, Abcb1b, Abcc1, Abcc2, Abcc3) and members of the organic anion-transporting polypeptides (Slco1a4, Slco2b1, Slco4a1) were down-regulated. A biodistribution study was carried out with glyburide, a hypoglycemic sulfonylurea substrate of both ABC efflux and Oatp uptake transporters. Although administration of endotoxin resulted in comparable plasma concentrations of glyburide, a pronounced increase in the accumulation of glyburide was seen in the fetuses of endotoxin-treated rats (162% of controls, \( p < 0.01 \)). Glyburide plasma protein binding was not affected by endotoxin treatment. Overall, our results demonstrated a significant reduction in the placental expression of several important drug transporters during endotoxin-induced inflammation. Alterations in glyburide distribution highlight the potential importance of both influx and efflux placental transporters in impacting fetal drug exposure.

Inflammation is a component of many common conditions encountered in pregnancy, including infection, preeclampsia, and gestational diabetes (Slaviero et al., 2003). The inflammatory response is associated with the induction of endogenous proinflammatory cytokines which impact gene regulation. Inflammation mediated either by vaccines, chemicals, cytokines or endotoxins has been shown to down-regulate several of the cytochrome P450 isoforms (Morgan, 1997; Renton, 2001; Slaviero et al., 2003). In addition to drug metabolism, inflammatory responses also affect drug distribution and clearance through changes in drug transporters. Indeed, a down-regulation in the expression and activity of several ATP-binding cassette (ABC) drug transporters, such as P-glycoprotein (Pgp or Mdr1) and the multidrug resistance-associated proteins (Mrp) have been reported in endotoxin, cytokine, and turpentine models of inflammation (Piqutte-Miller et al., 1998; Hartmann et al., 2001; Kalitsky-Szirtes et al., 2004). It has only been recognized recently that the breast cancer resistance protein (Bcrp/Abcg2), a member of the ABC superfamily of transporters, also plays an important role in drug distribution and clearance. However, very little is known regarding the impact of inflammation on the in vivo expression of Bcrp.

Bcrp, originally discovered in breast cancer cells with a multidrug resistance phenotype, is thought to play an important protective role in normal tissues (Doyle and Ross, 2003). Similar to other ABC transporters, Bcrp is a high-capacity efflux transporter with a large variety of substrates, including anticancer and anti-retroviral drugs, toxins, endogenous nutrients, hormones, and food additives (Staud and Pavek, 2005). Because Bcrp is highly expressed in placenta and is possibly involved in the placental transport of numerous compounds, the regulation and activity of Bcrp could be of critical importance for fetal development and safety. It has been reported that glyburide, a sulfonylurea drug for treating hyperglycemia, is a substrate of Bcrp (Gedeon et al., 2006). To date, more than 800 pregnant women have participated in clinical trials with glyburide, and its usage as first line therapy for gestational diabetes has been growing (Feig et al., 2007). Fetal distribution of glyburide was recently shown to be significantly limited by Bcrp1 in mice (Zhou et al., 2008). Other transporters, such as the organic anion-transporting polypeptides (Oatps), which belong to the solute carrier family, and Mrp3 have also been reported to interact with glyburide (Shitara et al., 2002; Gedeon et al., 2006).

Therefore, the primary objective of this study was to investigate the effect of endotoxin-induced inflammation on the placental expression of several key ABC drug efflux (Bcrp, Mdr1a, Mdr1b, Mrp1, Mrp2, and Mrp3) and solute carrier drug uptake (Oatp1a4, Oatp2b1,
Stranded cDNA was synthesized from 2.5 μg of RNA by use of the First Strand cDNA synthesis kit (MBI Fermentas, Hanover, MD) according to the manufacturer’s protocol. Amplification using real-time PCR was performed with the LC FastStart DNA Master SYBR Green I. The reaction was carried out by incubating with the LC FastStart DNA Master SYBR Green I solution. Tissue sections were analyzed using two-way ANOVA (GraphPad Software Inc., San Diego, CA). A difference in means with a p value of less than 0.05 was considered statistically significant.

Materials and Methods

Animals and Experimental Design. All animal studies were approved by the University of Toronto Animal Care Committee (protocol no. 2000-6622) and conducted in accordance with the guidelines of the Canadian Council on Animal Care. Pregnant Sprague-Dawley rats (gestational day 17; Charles River Laboratories, Saint-Constant, QC, Canada) were injected i.p. with single 0.1, 0.5, or 1.0 mg/kg doses of bacterial endotoxin lipopolysaccharide (from Escherichia coli serotype O55:B5; Sigma-Aldrich, St. Louis, MO) dissolved in saline. Control pregnant rats (gestational day 17) were injected with sterile saline. Animals were sacrificed at 6, 12, 18, or 24 h postinjection (n = 3–7/group). Placentas were immediately harvested and preserved in liquid nitrogen for mRNA and protein analyses. Another set of placentas was fixed in 4% paraformaldehyde for immunohistochemistry analysis. Maternal blood was collected, and plasma was obtained via centrifugation at 4°C and preserved at −80°C for measurement of cytokines.

Determination of Placental Transporter mRNA Expression. The mRNA levels of each transporter were examined by quantitative real-time PCR. Methods for RNA isolation, cDNA synthesis and real-time PCR have been reported (Wang et al., 2005). Briefly, RNA was extracted from tissues by use of the First Strand cDNA synthesis kit (MBI Fermentas, Hanover, MD) according to the manufacturer’s protocol. Amplification using real-time PCR was performed with the LC FastStart DNA Master SYBR Green I solution. Tissue sections were analyzed using two-way ANOVA (GraphPad Software Inc., San Diego, CA). A difference in means with a p value of less than 0.05 was considered statistically significant.

Western Blot Analysis. Total membrane protein was isolated as previously reported (Hartmann et al., 2001; 2002; Wang et al., 2005). Protein samples (20 μg) were separated on 10% SDS-polyacrylamide gels, transferred to a Hybond nitrocellulose membrane (Amersham), and blocked with Tris-buffered saline containing 0.05% Tween 20 and 5% nonfat milk at 4°C overnight. Membranes containing transporter protein were then cut in half, and the upper portion (molecular mass > 64 kDa, based on the Kalaedoscope Molecular weight Standards; Bio-Rad, Hercules, CA) was incubated with M-70 antibody, which recognizes rat Bcrp (1:500; Santa Cruz Biotechnology, Santa Cruz, CA) followed by horseradish peroxidase-conjugated anti-rabbit secondary antibody (1:2000; Santa Cruz Biotechnology). To control for variability in protein loading, the lower part of the membrane (molecular mass < 64 kDa) was incubated with anti-β-actin antibody (AC-15 (1:2000); Sigma-Aldrich) followed by horseradish peroxidase-conjugated anti-mouse secondary antibody (1:2000; Amersham). Bound antibody was detected using an Enhanced Chemiluminescence detection kit (Amersham) and visualized after exposure on Kodak BioMax-MS films (Eastman Kodak, Rochester, NY). Protein band intensity was quantified by Alpha Ease FC imaging software (Alpha Innotech, San Leandro, CA).

Biodistribution of Glyburide. Pregnant rats that had received 0.5 mg/kg endotoxin or saline (controls) 24 h earlier were used for the glyburide biodistribution study (n = 3–5 per group). Glyburide (24 μg/kilogram dissolved in ethanol and diluted in saline containing 25% dimethyl sulfoxide; Sigma-Aldrich) was administered i.v. through the tail vein. Animals were sacrificed 1 h after glyburide administration. The 1-h time period was chosen based on previous reports of glyburide biodistribution in pregnant rats and mice (Sivan et al., 1993; Zhou et al., 2008). Individual placentas and fetuses and other maternal organs (liver, intestine, kidney, and brain) were separated, snap-frozen in liquid nitrogen, and preserved at −80°C until use. Glyburide concentration in maternal plasma and in fetal and maternal tissues was determined by a validated HPLC-UV assay. Plasma protein binding of glyburide was measured in individual plasma samples obtained from endotoxin and control rats using Millipore CentriFresh devices (Millipore, Woodbridge, ON, Canada).

HPLC-UV Assay. Plasma and tissue glyburide concentrations were determined by a validated HPLC-UV assay (Gedeon et al., 2008). The assay was adapted for fetal and maternal tissue homogenates. Chromatography analysis was carried out using the Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) and a Waters 2487 Dual A Absorbance Detector (Waters, Milford, MA). The mobile phase consisted of acetonitrile/50 mM ammonium acetate buffer, pH 5.35 (55:45, v/v). Separation was performed at a flow rate of 1.0 ml/min on a Waters Xterra MS C18 column (4.6 × 250 mm, 5 μm), and the detection wavelength was set at 254 nm.

Tissue samples (approximately 0.3 g) were homogenized in double-distilled water (4.0 ml water/g tissue) using a sonic dismembrator (model 100; Fisher Scientific Co., Pittsburgh, PA). Plasma samples (600 μl) and tissue homogenates (600 μl) were vortexed with 8.0 ml of methyl butyl ether, shaken for 10 min, and centrifuged at 3750 rpm for 20 min. Organic phases were transferred into clean test tubes, evaporated dry under nitrogen, and reconstituted with 100 μl of the mobile phase.

Standard calibration curves were constructed by adding glyburide to drug-free plasma or tissue homogenates (600 μl) to give final concentrations ranging from 0.025 to 0.500 μg/ml. The detection sensitivity of this HPLC-UV assay was 4.2 ng/ml and 5.6 ng/g for plasma and tissue samples, respectively.

Immunohistochemistry. Placental tissues fixed in 4% paraformaldehyde were paraffin-embedded and cut into 5-μm sections. Tissue sections were deparaffinized in xylene and rehydrated. Endogenous peroxidase activity was blocked using 0.3% H2O2 in methanol for 20 min. Before staining, paraffin sections were preheated in a microwave. A rabbit anti-Bcrp antibody M-70 (Santa Cruz Biotechnology) was incubated with the sections. The slides were then incubated for 40 min with a Nova Red-conjugated goat anti-rabbit antibody (1:400; University Health Network, Toronto, ON, Canada). After staining, the slides were visualized under a microscope, and areas of interest were recorded by a digital camera.

Measurement of Plasma Cytokine Levels. Maternal plasma cytokine levels of tumor necrosis factor (TNF-α and interleukin (IL)-6 were determined by using an enzyme-linked immunosorbent assay (ELISA) at 6 and 24 h postendotoxin administration. ELISA protocols for TNF-α (Abcam Inc., Cambridge, MA) and IL-6 (R&D Systems, Minneapolis, MN) were followed according to manufacturer’s instructions. The samples were examined in duplicate, and results within the standard curve range are reported.

Data and Statistical Analysis. A two-tailed Student’s t-test was employed for statistical comparison of biodistribution and ELISA results between the endotoxin and control groups. For analysis of dose effects and comparison with controls at each time point, a one-way analysis of variance (ANOVA) was applied followed by the Newman-Keuls multiple comparison test for post hoc analysis. Multiple comparisons of data for all dose and time points were subsequently analyzed using two-way ANOVA (GraphPad Software Inc., San Diego, CA). A difference in means with a p value of less than 0.05 was considered statistically significant.

Table 1: Primers used for real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
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<tr>
<td>gapdh</td>
<td>5′-CCATCACCACCATCCTCCAGG-3′</td>
<td>5′-CCTGCTTCACCCACCTTCCTG-3′</td>
</tr>
<tr>
<td>bcrp</td>
<td>5′-AGTCCGGAAAACACGCTGGA-3′</td>
<td>5′-CCCCATCAACGCTGCTCCTTG-3′</td>
</tr>
<tr>
<td>mdr1a</td>
<td>5′-GACGGATTGTAATGGTGACCA-3′</td>
<td>5′-AAGGATCGGAAAGAATAA-3′</td>
</tr>
<tr>
<td>mdr1b</td>
<td>5′-GGTTCACATGTTGAGTGATCT-3′</td>
<td>5′-GGTTCATGGTCGTCGTCTCTTGA-3′</td>
</tr>
<tr>
<td>mpt1</td>
<td>5′-TTCTAGTGTGAGCAGGCTGT-3′</td>
<td>5′-TTGGGACATGTCATAAGAAGACG-3′</td>
</tr>
<tr>
<td>mpt2</td>
<td>5′-GGTACCAGGTCTCTCCTCTG-3′</td>
<td>5′-GGGCAAGGACCCTGTTTGAGTC-3′</td>
</tr>
<tr>
<td>mpt3</td>
<td>5′-GGTGCTGAAGAATGGTCATCT-3′</td>
<td>5′-GGACCAGGACCCTGTTTGAGTC-3′</td>
</tr>
<tr>
<td>oatpl4</td>
<td>5′-GGTGTGTTGTTGATGCAGTTG-3′</td>
<td>5′-GGCATAATGCTTCATTCTCCTGT-3′</td>
</tr>
<tr>
<td>oatp2b1</td>
<td>5′-GACTATGCGTCCACCTGGTCTG-3′</td>
<td>5′-GGTGGTCTAGTGGTTCGCAAGATG-3′</td>
</tr>
<tr>
<td>oatp4a1</td>
<td>5′-CTGGGAGCTCGTCTGACTAAT-3′</td>
<td>5′-GGCGGAATGTCGACTTGCAGG-3′</td>
</tr>
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</table>
We observed a significant dose-dependent down-regulation of Bcrp at 18 to 24 h in placentas obtained from endotoxin-treated rats (Fig. 1A). A corresponding decrease in immunodetectable levels of Bcrp protein was seen in placenta isolated at 24 h (Fig. 1B). Immunohistochemical analysis detected high levels of Bcrp expression on the trophoblast cells of endotoxin-treated rats. A significant dose- and time-dependent down-regulation of Bcrp expression was also seen in the endothelium of some fetal capillaries.

Expression of Bcrp, both on the trophoblasts and fetal capillaries, was noticeably diminished in endotoxin-treated animals (Fig. 2, D–F) as compared with saline-treated animals (Fig. 2, A–C).

As depicted in Fig. 3A, we observed a pronounced decrease in the placental mRNA expression of Mdr1a (Abcb1a) in the endotoxin-treated rats. A significant dose- and time-dependent down-regulation was seen from 18 to 24 h postendotoxin injection (p < 0.05). Endotoxin administration also imposed a significant decrease in mRNA levels of Mdr1b (Abcb1b) at 18 and 24 h (Fig. 3B).

Significant decreases in the mRNA levels of Mrp1 (Abcc1), Mrp2 (Abcc2), and Mrp3 (Abcc3) were seen in the endotoxin-treated rats (Fig. 4). Down-regulation of Mrp1 mRNA was observed as early as 6 h postendotoxin injection, with a profound dose- and time-dependent effect seen at 24 h (Fig. 4A). Decreases in mRNA levels of Mrp2 were seen between 12 and 24 h in the endotoxin-treated rats, but this was only significant at 12 and 18 h (Fig. 4B). Significant decreases in mRNA levels of Mrp3 were seen between 18 and 24 h in the endotoxin-treated rats (Fig. 4C).

Because rat Oatp1a4 and human OATP2B1 have been reported to interact with glyburide, we also examined the impact of endotoxin on the expression of several Oatp isoforms. The mRNA levels of the predominant isoforms of placental influx transporter Oatp, which include Oatp1a4 (Slco1a4; also known as Oatp2), Oatp2b1 (Slco2b1; also known as Oatp-B), and Oatp4a1 (Slco4a1; also known as Oatp-E) (Mikkaichi et al., 2004), were analyzed. In general, endotoxin-mediated reduction in mRNA levels of the Oatp isoforms occurred at earlier time points and recovered by 24 h postendotoxin administration (Fig. 5). As compared with controls, Oatp1a4 mRNA was significantly reduced at 6 to 12 h in the endotoxin-treated rats (Fig. 5A), whereas significant decreases in Oatp2b1 and Oatp4a1 mRNA were seen between 12 and 18 h (Fig. 5, B and C) (p < 0.05).

The biodistribution of glyburide was examined to assess the potential impact of inflammation-mediated changes in the expression of transmembrane transporters. The biodistribution of glyburide was examined to assess the potential impact of inflammation-mediated changes in the expression of transmembrane transporters.
porters on fetal drug exposure. Although glyburide levels tended to be higher in control as compared with endotoxin-treated rats at 1 h postadministration, these differences did not reach statistical significance (control, 166 ± 48 ng/ml; endotoxin, 77 ± 13 ng/ml). However, a significant increase of glyburide accumulation was seen in fetuses of endotoxin-treated rats (162% of saline, p < 0.01). Normalizing fetal tissue glyburide levels to maternal plasma levels also revealed a 3.5-fold increase in the glyburide concentration ratio for fetuses obtained from endotoxin-treated rats as opposed to those from saline controls (p < 0.001) (Fig. 6). Although absolute amounts of glyburide accumulation (nanograms per gram of tissue) were significantly different only in the fetal tissue, normalized tissue/plasma levels of glyburide were significantly higher in maternal tissues obtained from endotoxin-treated rats. In the placenta, glyburide concentration ratio was 2.5-fold higher in endotoxin-treated rats (p < 0.01), whereas the difference was 1.7 to 2.9-fold higher in maternal liver, intestine, kidney, and brain (p < 0.05) (Fig. 6). Plasma protein binding of glyburide was not significantly different between control and endotoxin groups (control, 80 ± 1.6%; endotoxin, 82 ± 1.6%).

Maternal Plasma Cytokine Levels after Endotoxin Administration. In vivo administration of endotoxin is known to induce plasma levels of the proinflammatory cytokines. TNF-α and IL-6 are two of the major cytokines induced upon endotoxin administration in the circulation system of rodents. Our ELISA results demonstrated a dose-dependent 2- to 26-fold induction in maternal plasma TNF-α levels at 6 h postendotoxin administration, with levels subsiding by 24 h (Table 2). At 6 h, there was a pronounced 3- to 4-fold elevation in the plasma IL-6 levels of rats that received 0.5 to 1.0 mg/kg endotoxin (Table 2). By 24 h, plasma IL-6 levels decreased in rats treated with the lower endotoxin doses, but levels were dramatically elevated in the 1.0 mg/kg endotoxin treatment group (Table 2).

Discussion

A wide array of pathological, physiological, and hormonal changes occur in pregnancy, which can significantly impact the pharmacokinetics of drugs (Anger and Piquette-Miller, 2008). Overall, our findings demonstrated that endotoxin-induced inflammation imposes a pronounced decrease in the placental expression of several key drug transporters in pregnant rats. Although the temporal pattern and magnitude of endotoxin-mediated changes differed between transporters, a down-regulation was seen in mRNA levels of both efflux and influx transporters, including Bcrp, Mdr1a, Mdr1b, Mrp1, Mrp2, Mrp3, Oatp1a4, Oatp2b1, and Oatp4a1. It is important to note that these particular transporters influence the transplacental passage of numerous clinically important drugs and toxins; therefore, alterations in their expression and activity have the potential to greatly impact fetal
Dependent reduction in Bcrp mRNA levels 18 to 24 h after endotoxin administration, with a corresponding decrease in the immunodetectable levels of protein. Interestingly, a down-regulation in Bcrp expression was detected in both the placental trophoblast cells and fetal capillary endothelium of endotoxin-treated animals. A decrease in the in vitro expression of Bcrp in primary term trophoblasts was recently reported after exposure to TNF-α or IL-1β (Evseenko et al., 2007). Consistent with these in vitro findings, our results demonstrate a significant induction of TNF-α and IL-6 in the maternal plasma of endotoxin-treated rats. This suggests that these cytokines could be involved as mediators of down-regulation of placental Bcrp in endotoxin-treated rats.

Bcrp has been shown to restrict the passage of topotecan and mitoxantrone to the fetus in pregnant mice; thus, it is believed that Bcrp plays an important role in the protection of fetus from exposure to toxic chemicals (Jonker et al., 2000). To investigate the impact of endotoxin-mediated down-regulation of transporter expression on fetal drug exposure, we conducted a biodistribution study of glyburide, a sulfonylurea hypoglycemic agent that can be used to manage gestational diabetes. Glyburide has been shown to be actively transported by the human isoforms of BCRP and MRP3 but not PGP, MRP1, or MRP2 (Gedeon et al., 2006). Furthermore, it has recently been shown that fetal distribution of glyburide is increased in Bcrp1−/− pregnant mice as compared with wild-type controls (Zhou et al., 2008). Because Bcrp is involved in the active efflux of glyburide, we anticipated that down-regulation of Bcrp in endotoxin-treated rats would result in increased fetal exposure. Indeed, we observed increased absolute and plasma normalized concentrations of glyburide in the fetuses of endotoxin-treated rats. Hence, inflammation-mediated changes in the expression of Bcrp appear to impact the overall fetal exposure of glyburide. Although we cannot rule out the possibility that glyburide distribution at other time points could reveal additional changes, previous studies have reported a time delay in glyburide distribution to the fetus, with concentrations reaching a maximum at 1 h (Sivan et al., 1995; Zhou et al., 2008). Moreover, because concentrations of glyburide in fetal tissues decrease in parallel to maternal plasma concentrations after initial distribution, fetal/maternal plasma concentration ratios have been found to remain relatively constant between 1 and 4 h after glyburide administration. Therefore, it is probable that endotoxin-mediated changes in the fetal accumulation of glyburide are likely to persist at later time points. Glyburide concentrations were comparable with controls in maternal tissues, but when normalized to

exposure to xenobiotics. Because there are many maternal conditions that are associated with an inflammatory response, this could represent an important source of variability in fetal drug exposure and teratogenicity.

Placental efflux transporters, such as Bcrp and Pgp, are believed to protect placental and fetal tissues by removing potentially toxic xenobiotics and endogenous metabolites. It has been previously demonstrated that endotoxin and other inflammatory stimuli impose a down-regulation of Pgp in epithelial tissues of the liver, intestine, brain, and placenta in rodents (Piquette-Miller et al., 1998; Hartmann et al., 2001; Goralski et al., 2003; Kalitsky-Szirtes et al., 2004; Wang et al., 2005). Recently, it has been reported that intestinal expression of BCRP is decreased in patients with ulcerative colitis (Englund et al., 2007); however, the effect of inflammation on the placental Bcrp expression is unknown. Bcrp is highly expressed in the apical syncytio-trophoblast membrane and fetal capillary endothelium of human term placenta (Maliepaard et al., 2001; Litman et al., 2002; Ceckova-Novotna et al., 2006). Our results demonstrated a significant dose-dependent reduction in Bcrp mRNA levels 18 to 24 h after endotoxin administration, with a corresponding decrease in the immunodetectable levels of protein. Interestingly, a down-regulation in Bcrp expression was detected in both the placental trophoblast cells and fetal capillary endothelium of endotoxin-treated animals. A decrease in the in vitro expression of Bcrp in primary term trophoblasts was recently reported after exposure to TNF-α or IL-1β (Evseenko et al., 2007). Consistent with these in vitro findings, our results demonstrate a significant induction of TNF-α and IL-6 in the maternal plasma of endotoxin-treated rats. This suggests that these cytokines could be involved as mediators of down-regulation of placental Bcrp in endotoxin-treated rats.

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placental samples obtained from either control or endotoxin treated rats. Some of these changes could result, in part, because of Bcrp down-regulation in maternal tissues; however, changes in Bcrp alone cannot fully explain these findings. Although alterations in plasma protein binding could result in an increased tissue distribution, we did not detect significant differences in glyburide protein binding in plasma obtained from endotoxin and control rats.

Because evidence exists suggesting that glyburide uptake may occur through several of the Oatp transporters, it is plausible that inflammation-mediated changes in these transporters could contribute to alterations in glyburide uptake and distribution. Glyburide has been shown to be transported by human OATP2B1 (Satoh et al., 2005) and can inhibit transport activity of rat Oatp1a4 at high concentrations (Shitara et al., 2002). There are several Oatp isoforms expressed in rat placenta, particularly Oatp2b1, Oatp4a1, and, to a lesser extent, Oatp1a4 (St-Pierre et al., 2004). We detected significantly lower mRNA levels of all three isoforms in placental tissues obtained from endotoxin-treated rats. Likewise, previous studies have reported an endotoxin-mediated down-regulation of Oatp1a4 mRNA in the brain and liver of rodents (Hartmann et al., 2002; Goralski et al., 2003). Because a down-regulation of the Oatp transporters would serve to decrease, rather than increase, the placental uptake and fetal accumulation of glyburide, it does not appear that in vivo changes in the placental expression of the Oatp transporters play a major role in the fetal accumulation of glyburide.

In summary, our results demonstrate that endotoxin-induced inflammation significantly down-regulates the expression of several important drug transporters in placenta. This includes transporters that are involved in both placental drug uptake and drug efflux. Moreover, we observed a significant increase in the distribution of glyburide into fetal tissues in endotoxin-treated rats. Because this drug is a substrate of both influx and efflux transporters, our findings highlight the importance of considering the mutual functioning relationship of these transporters, rather than the changes in only a single group of transporters. Different substrates are likely to have different affinities for the transporters that direct their transplacental transfer; thus, the interaction between inflammatory stimuli and transporter activities should be assessed when considering medication usage during pregnancy.

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References


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