Impact of Polyinosinic/Polyctydidylic Acid on Placental and Hepatobiliary Drug Transporters in Pregnant Rats

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ABSTRACT:
Although inflammation is known to impose changes in the expression and activity of drug transporters, little is known about the impact of inflammatory stimuli on these transporters during pregnancy. Our objective was to study the effect of viral-induced inflammation on key maternal hepatic and placental drug transporters and their endogenous substrates. Acute inflammation was induced in pregnant Sprague-Dawley rats (gestation day 17–18, n = 5–6/group) by single intraperitoneal doses of polyinosinic/polyctydidylic acid [poly(I:C)] (2.5 or 5.0 mg/kg) with saline as a control. Tissues were harvested 24 h later. Expression of transporters was measured via real-time polymerase chain reaction and Western blotting. Maternal plasma levels of cytokines, bile acids, and bilirubin and fetal levels of bile acids were examined. Plasma concentrations of interferon-γ, tumor necrosis factor-α, and interleukin-6 were significantly induced in poly(I:C)-treated rats, compared with controls (p < 0.001). Significant down-regulation of placental Abcc1a/b, Abcc3, Abcg2, Slco1a4, and Slco4a1 mRNA and of hepatic Abcc2, Abcg2, Slco1a4, Slc10a1, and Cyp3a2 mRNA was observed in poly(I:C)-treated rats. Hepatic Abcb1b and Abcc3 mRNA levels were significantly induced. Hepatic protein levels of P-glycoprotein, multidrug resistance-associated protein 2, and breast cancer resistance protein were significantly down-regulated relative to those for controls (p < 0.05). Total bile acids in maternal plasma were significantly increased at the higher dose of poly(I:C). In summary, the poly(I:C) model of viral infection imposes significant changes in the expression of key drug transporters in placental and hepatic tissues of pregnant rats. Because many clinically important endogenous and exogenous compounds are substrates of these transporters, inflammation-mediated changes in transporter expression could affect their maternal disposition and fetal exposure.

Introduction
Membrane transport proteins are of critical importance in the cellular uptake and efflux of numerous endogenous and xenobiotic compounds in the body. Pharmacokinetic processes often involve active transport of substrates across epithelial membranes, and this frequently involves members of the ATP-binding cassette (ABC) transporter family. ABC proteins are efflux transporters, several of which are known to move many clinically important drugs, as well as endogenous substrates such as biliary compounds. P-glycoprotein (P-gp) (encoded by ABCB1 in humans and Abcb1a and Abcb1b in rodents), several of the multidrug resistance-associated proteins (MRPs) (encoded by Abcc genes) and the breast cancer resistance protein (Bcrp) (encoded by Abcg2) are of particular importance in this regard. These drug transporters are highly expressed in the epithelium of the liver, gastrointestinal tract, brain, and placenta (Schinkel and Jonker, 2003).

The placenta plays an integral role in the protection of the fetus from potentially harmful xenobiotics and endogenous substrates that may be found in the maternal circulation. Moreover, because the fetal hepatobiliary system is not fully developed during gestation, the placenta also plays a crucial role in detoxification of cholephilic compounds during intrauterine life (Macias et al., 2009). Key placental ABC drug transporters are believed to aid in the efflux or removal of such endogenous substrates as well as other exogenous substrates, thus serving a protective role in this tissue. Dramatically increased accumulation of P-gp substrates (digoxin, saquinavir, and paclitaxel) in the fetus of P-gp-deficient mice has been demonstrated (Smit et al., 1999). Likewise, studies in Bcrp1(−/−) mice demonstrated a significant increase in fetal accumulation of Bcrp substrates (nitrofurantoin and glubride) compared with that in wild-type mice (Zhang et al., 2007; Zhou et al., 2008).

It is well established that a number of inflammation-inducing agents, such as vaccines, chemicals, cytokines, or endotoxins can lead to changes in drug metabolism and disposition by down-regulating cytochrome P450-metabolizing enzymes (Morgan, 1997; Renton, 2001). An ever-increasing amount of data now also points to the involvement of drug-transporting proteins in the phenomenon of...
inflammation-mediated changes in drug disposition, a process thought to be regulated by proinflammatory mediators and nuclear hormone receptors (Petrovic et al., 2007; Morgan et al., 2008; Teng and Piquette-Miller, 2008). Previous studies have demonstrated that an acute inflammatory response induced by bacterial endotoxin [lipo-polysaccharide (LPS)] down-regulates the expression and activity of key ABC transporters in rodent liver, brain, renal, intestinal, and placental tissues with a corresponding change in drug disposition (for review, see Petrovic et al., 2007; Morgan et al., 2008). In addition, other disease models associated with inflammation, such as induced chronic renal failure, diabetes, cholestasis, or cancer, have been shown to modulate the hepatic expression of several transporters (Slaviero et al., 2003; Teng and Piquette-Miller, 2007; Nolin et al., 2008; Anger et al., 2009; Yang et al., 2009).

Viral infections are a significant concern in pregnancy. Many women may be affected by some form of a viral infection throughout the course of their gestation. Whereas some are fairly mild and easily treatable, infections can range in severity to include influenza, herpes viruses, and hepatitis B virus, as well as cytomegalovirus and human immunodeficiency virus infections, which are still widespread in many parts of the world. Viral infections are commonly modeled in vivo by administration of a synthetic viral-like double-stranded (ds) RNA, polyinosinic/polycytidylic acid [poly(IC)]. Best known as an inducer of interferon (IFN), poly(IC) is also associated with the induction of other proinflammatory cytokines such as interleukin (IL)-6, IL-10, IL-12, and tumor necrosis factor (TNF-α), as well as with the induction of an acute phase response (Fortier et al., 2004). Poly(IC) acts via Toll-like receptor (TLR) 3 and TLR2 pathways and elicits an inflammatory response through a cytokine cascade that is partially different from that for bacterial infections, which are commonly modeled by the administration of LPS, which acts via TLR4 (Vercammen et al., 2008; Barbalat et al., 2009). Because very little information exists as to the effects of viral infection on the expression of drug transporters, our objective was to examine the expression of several key drug transporters in livers and placentas of poly(IC)-treated pregnant rats. Furthermore, because inflammation can mediate the disturbance of maternal hepatobiliary homeostasis, which can affect fetal outcomes, we also examined several important bile acid uptake transporters, as well as the organic anion-transporting polypeptide (Oatps/Slc21a) and the sodium taurocholate-cotransporting polypeptide (Ntcp/Slc10a1), and examined levels of biliary substrates.

Materials and Methods

Animals and Experimental Design. Pregnant near-term Sprague-Dawley rats [gestational day (GD) 17–18; Charles River Laboratories, Senneville, QC, Canada] received intraperitoneal injections of single 2.5 or 5.0 mg/kg doses of poly(IC) (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) dissolved in phosphate-buffered saline or with a single 0.5 mg/kg dose of bacterial LPS (Escherichia coli serotype O55:B5; Sigma-Aldrich, St. Louis, MO) dissolved in saline. Control pregnant rats (GD 17–18) were given sterile saline injections. Animals were sacrificed at 24 h after injection (n = 4–7/group). Maternal livers, placentas, and fetuses were immediately harvested and preserved at nitrogen, redissolved in 2 ml of ethanol, and centrifuge-filtered using a modified procedure from Lee et al. (2008). Three to five fetuses from the same mother were pooled together for analysis. In brief, thawed fetal livers including the entire gastrointestinal tract were placed in 50 ml of ethanol, minced well, and boiled down to approximately 20 ml. Samples were filtered through No. 2 Whatman paper, and the volume was brought to 25 ml. Samples were analyzed directly, whereas fetal BAs were first extracted with ethanol using a modified procedure from Lee et al. (2008).
Results

Cytokine Response to Poly(I:C). Proinflammatory cytokines were examined in maternal plasma samples from poly(I:C)-treated and vehicle control rats. Administration of stress stimuli such as poly(I:C) is known to trigger an acute-phase reaction and a release of cytokines. Indeed, our ELISA results demonstrated a significant induction of IFN-γ 24 h after administration of 2.5 or 5.0 mg/kg poly(I:C) compared with controls (Fig. 1A). Measurements of IFN-γ in control samples were below detection limits and were thus considered to be <10 pg/ml. Furthermore, IL-6 and TNF-α levels were also increased compared with controls, with significant differences at the 5.0 mg/kg poly(I:C) dose (Fig. 1, B and C).

Effect of Poly(I:C) and LPS on Placental and Hepatic mRNA. The impact of poly(I:C) on the expression of transporters in placental and hepatic tissues was examined by qRT-PCR. In placenta, we observed significant changes in the mRNA expression of several transporters in the liver (Fig. 2, A and B). Placental Abcb1a and Abcb1b were significantly down-regulated at one or both doses of poly(I:C) to approximately half of the value of control mRNA levels (p < 0.01). Expression of Abcg2 was also significantly decreased to 51 ± 9% of control, at the higher poly(I:C) dose (p < 0.05). Although Abcc1 and Abcc3 were significantly down-regulated at both doses of poly(I:C) to 54 to 70% of control, at the higher poly(I:C) dose (p < 0.01), we did not observe significant changes in Abcc2 mRNA levels at either dose. Compared with controls, placental mRNA levels of Slco1a4 and Slco4a1 were significantly reduced in the poly(I:C)-treated rats, with values ranging from 53 to 77% of controls (p < 0.05). No changes were observed in the placental mRNA expression of Slco2b1.

Administration of poly(I:C) also imposed significant changes in the expression of several transporters in the liver (Fig. 3). After administration of 5.0 mg/kg poly(I:C), Abcg2 and Abcc2 were significantly reduced to 20 ± 5 and 31 ± 6% of control values, respectively, whereas Abcc3 was increased to 305 ± 67% of controls (p < 0.05). Although we did not observe any changes in Abcb1a mRNA levels, we found a pronounced up-regulation of Abcb1b (p < 0.01). Significant down-regulation at both doses of poly(I:C) was seen for Slc10a1 (Ntcp) and Slc10a4 (Oatp1a4, also known as Oatp2) genes, with values ranging from 8 to 38% of controls (p < 0.05). Expression of hepatic Abcb11 (bile salt export pump) was reduced to 54 to 70% of control levels. In addition, the hepatic metabolizing enzyme Cyp3a2a was significantly decreased to 24 ± 2% of the control mRNA value (p < 0.05).

Because LPS-mediated changes in hepatic expression have not been previously described in pregnant rats, we also examined the poly(I:C) response in LPS-exposed pregnant rats.
impact of LPS on the hepatic mRNA expression of several transporters (Fig. 4). Changes seen in the LPS model were consistent with those in the poly(I:C) model, with the exception of Abcb1a, which was significantly down-regulated in LPS- but not poly(I:C)-treated pregnant rats. Moreover, at 24 h, levels of Abcg2 and Cyp3a2 were decreased to 65 and 55% of controls, respectively, but did not reach statistical significance. However, at 6 h after LPS administration, Abcg2 was significantly down-regulated to 47% of controls (0.5 mg/kg LPS, n = 3, p < 0.05), and Cyp3a2 was significantly reduced to 40% (1.0 mg/kg LPS, n = 3, p < 0.05).

**Effect of Poly(I:C) on Placental and Hepatic ABC Transport Proteins.** Relative to controls, immunodetectable levels of P-gp, Mrp2, and Bcrp were significantly down-regulated in the livers of poly(I:C)-treated rats at both doses (p < 0.05) (Fig. 5A). P-gp was significantly reduced to 17 to 49% of control levels, Bcrp was reduced to 29 to 58% of control levels, and Mrp2 was reduced to 45 to 59% of control values. We did not find differences in placental P-gp or Bcrp expression between the control group and the poly(I:C)-treated groups (Fig. 5B); however, the immunodetectable levels in our placental crude membrane preps were very low, with several samples below detection limits.

**Effect of Poly(I:C) on Maternal and Fetal Concentrations of Biliary Compounds.** Because poly(I:C)-treated rats demonstrated changes in the expression of many of the major hepatic bile acid transporters (Abcb11, Abcc2, Abcc3, Slc10a1, and Slc10a4), we examined maternal and fetal levels of BAs. After 24 h of administering 5 mg/kg poly(I:C), total BA levels in maternal plasma were dramatically increased (8-fold, p < 0.001) compared with plasma levels of vehicle control rats (Fig. 6A). After normalizing to tissue weight of pooled fetuses, we also observed a trend toward increased magnitudes of total BAs in fetuses from poly(I:C)-treated mothers, compared with controls, but this did not reach statistical significance, possibly because of high intergroup variation (Fig. 6B). On the contrary, maternal plasma levels of direct and total bilirubin did not significantly differ between control and poly(I:C) groups (Fig. 6, C and D).

**Discussion**

Pregnancy is associated with a number of physiological and hormonal changes that can alter drug pharmacokinetics by modulating the mechanisms involved in drug uptake, metabolism, and excretion (Anger and Piquette-Miller, 2008). A further complication may be introduced with the onset of an acute or chronic inflammatory condition in the mother. Inflammation is not uncommon in pregnancy, and the induction of proinflammatory cytokines is seen with many prevalent obstetric complications including infections, chorioamnionitis, cholestasis of pregnancy, gestational diabetes, and preeclampsia (Romero et al., 2007). Inflammation-mediated changes in drug-metabolizing enzymes and drug-transferring proteins have been reported previously (for review, see Petrovic et al., 2007; Morgan et al., 2008); however, very little is known about the effects of the inflammatory response in pregnancy. We investigated the effect of viral and bacterial models of
inflammation on the placental and hepatic expression of several ABC drug efflux and solute carrier uptake transporters, which are known to transport numerous clinically relevant endogenous substrates and xenobiotics. Overall, our findings demonstrated that the induction of a viral-like response by poly(I:C) imposed a down-regulation in the expression of a number of key transporters involved in drug and bile acid transport in the liver and placenta of pregnant rats. Furthermore, significantly increased levels of total bile acids in maternal plasma of poly(I:C)-treated rats suggest functional changes in the activity of these transporters.

One of the most frequently used and best characterized pathogen-associated molecular patterns for simulating the effects of infection and systemic inflammation is the bacterial endotoxin, LPS. LPS-induced inflammation has been shown to affect the expression of placental transporters and fetal drug exposure (Petrovic et al., 2008). However, the endotoxin model may not be reflective of changes that occur during viral infections. Viruses typically produce dsRNA during their replication and are thus modeled by synthetic viral-like forms of dsRNA, such as poly(I:C). Bacteria and viruses bind to different TLRs, which can activate different signaling pathways and cytokine cascades. LPS binds to TLR4, whereas poly(I:C) binds primarily to TLR3 (Vercammen et al., 2008). Differences in the profile and rate of cytokine induction between the two pathogens have been reported (Lee et al., 2007; Reimer et al., 2008). Furthermore, whereas LPS mediates its effects through interferon regulatory factor 3 and nuclear factor-κB signaling, poly(I:C) can trigger a robust type I IFN response without activating those signaling pathways (Reimer et al., 2008). Because of such evidence of differential response pathways in bacterial and viral infections, we felt it was imperative to examine the effect of viral-like infection on the expression of drug transporters that are known to be involved in the disposition of clinically relevant substrates.

Overall, we found that poly(I:C) caused a significant down-regulation of several key ABC efflux and Oatp uptake transporters in the placenta. It is likely that the observed poly(I:C)-mediated induction of IFN-γ, TNF-α, and IL-6 is involved in the down-regulation of these placental transporters. Indeed, in vitro studies in human trophoblasts have reported cytokine-mediated changes in the expression of P-gp and Bcrp (Evseenko et al., 2007). In general, the down-regulation of ABC efflux and Oatp uptake transporters in placenta is consistent with changes seen in endotoxin-treated rats (Wang et al., 2005; Petrovic et al., 2008). Likewise, a dose-dependent decrease in the placental expression of Abcb1a has been reported in endotoxin-treated mice (Chen et al., 2005). In contrast, changes at the protein level differed between models of viral and bacterial infection. Although LPS mediates a significant down-regulation in the protein expression of Bcrp and P-gp in placenta (Petrovic et al., 2008), significant changes in protein expression were not detected in the poly(I:C)-treated rats. The protein expression of P-gp was not significantly changed; thus, the biological significance of the Abcb1a and Abcb1b down-regulation mediated by poly(I:C) remains to be elucidated. Because the effects of LPS were apparent only at a high dose, it is possible that we would see changes in placental protein expression with higher doses of poly(I:C). The maximal reduction of cytochrome P450 enzymes has been reported 24 h after the administration of a 10 mg/kg poly(I:C) dose (Anari et al., 1995). However, this dose is not tolerated well by pregnant rats and thus could not be examined in the present study.

We also examined the expression profile of important hepatic drug transporters in pregnant rats. Similar to LPS, poly(I:C) mediated a significant decrease in the expression of hepatic Bcrp in pregnant dams, both at the gene and protein level. However, down-regulation of Abcg2 seems to occur more rapidly after LPS administration as we observed a decrease at 6 h after LPS treatment but not at 24 h. The temporal pattern of gene expression changes in poly(I:C)-treated rats may differ from that seen after LPS administration because of quan-
titative or time-dependent differences in cytokine release, which can stimulate alternate cell signaling pathways. Although the effect of viral or bacterial models of inflammation on hepatic Bcrp expression has not been previously examined in pregnancy, a decrease in hepatic Bcrp expression has been reported in male rats with extrahepatic cholestasis (Villanueva et al., 2008; Bracakova et al., 2009). On the other hand, in a model of LPS-induced cholestasis, a 5-fold increase in the hepatic expression of Abcc2 mRNA was recently reported in male rats, accompanied by no changes in Bcrp protein expression (Bracakova et al., 2009). Therefore, it is likely that a complex interplay of cytokines is involved in the regulation of Bcrp. Indeed, in vitro studies in primary human hepatocytes reported that IL-6 mediates a decrease, whereas TNF-α mediates an increase in Bcrp expression (Vee et al., 2009).

Down-regulation of Abcc2/Mrp2 and induction of Abcc3 were seen in the livers of both poly(I:C)- and LPS-treated pregnant rats. Because Mrp2 and Mrp3 are believed to have a compensatory relationship in hepatocytes, this down-regulation is consistent with studies performed in nonpregnant rats (Cherrington et al., 2004; Donner et al., 2004). We also observed endotoxin-mediated down-regulation of Abcb1a and a strong induction of Abcb1b in pregnant rats. In line with our observations, previous studies in nonpregnant endotoxic rats have suggested differential regulation of these two hepatic P-gp gene isoforms (Cherrington et al., 2004; Wang et al., 2005). In contrast, administration of poly(I:C) did not mediate a strong change in Abcb1a; it led to a drastic induction of Abcb1b, but an overall decrease rather than an increase in P-gp protein expression. There is still debate as to the biological significance of the 1b gene isoform of P-gp, but our results indicate that mRNA levels of Abcb1b may not greatly contribute to protein levels of P-gp in rat liver. It is also possible that P-gp expression is regulated by additional post-transcriptional mechanisms activated within this viral model of infection. These could account for the discrepancies between the gene and protein expression of P-gp. Such a phenomenon has been observed previously in case of Mrp2 and bile salt export pump in human liver (Elferink et al., 2004). It is interesting that the expression of Abcb1b was down-regulated in placenta and induced in liver. Organ-specific regulation of Abcb1b has been reported previously in LPS-treated mice (Hartmann et al., 2005).

Because many of the affected transporters are also involved in bile acid transport, we examined maternal and fetal concentrations of biliary compounds. Our results demonstrated that the concentration of total bile acids in maternal plasma was significantly increased in poly(I:C)-treated rats relative to that in controls, probably as a consequence of inflammation-mediated changes in transporter expression. The mechanisms for hepatocyte regulation of bile acids are well known (Wolkoff and Cohen, 2003). The poly(I:C)-mediated reduction of hepatic Ntcp (Slc10a1) and Oatp2 (Slco1a4) probably contributed to a decrease in the basolateral uptake of bile acids, whereas decreased levels of the canalicular efflux transporters Mrp2 and P-gp could have resulted in decreased efflux into bile. In addition, because the basolateral transporter Mrp3 is also capable of effluxing bile acids from hepatocytes, its increased expression may have contributed to greater efflux into blood (Donner et al., 2004). Increased levels of total bile acids have been reported in pregnant rats with obstructive cholestasis (Hassan and Subbiah, 1980; Serrano et al., 2003). Likewise, studies in cholestatic nonpregnant rodents also reported an increase in total bile acids (Teng and Piquette-Miller, 2007; Yang et al., 2009). Repeated poly(I:C) administration over 28 weeks has been reported to induce the development of primary biliary cirrhosis in mice (Okada et al., 2005); however, it is unknown whether single doses of poly(I:C) can induce cholestasis.

Of note, we found that although there was a trend toward increased fetal bile acid levels, this did not reach statistical significance. During the intrauterine period, the placenta plays an important role in fetal hepatobiliary-like function (Macias et al., 2009). Because the Oatp uptake transporters were down-regulated in placentas of poly(I:C)-treated dams and placental Mrp2 efflux transporter was unchanged, it is possible that decreased uptake of bile acids could have played a protective role. Mrp3 levels were also reduced; however, the impact of this change is unclear because directionality of Mrp3 transport in the fetoplacental unit has not been definitively determined. Although we are not aware of any other studies examining the effect of inflammation on fetal bile acid levels, maternal cholestasis studies have yielded conflicting results, because both decreases and increases in rat fetal bile acids have been reported (Hassan and Subbiah, 1980; Serrano et al., 2003). Given that changes in fetal bile acid levels can lead to developmental challenges, a further elucidation of the effects of inflammation on fetal biliary transport is necessary.

Last, administration of poly(I:C) did not elicit significant changes in maternal plasma concentrations of bilirubin, which is transported via pathways similar to those transporting bile acids. We detected mildly increased plasma concentrations of both direct and total bilirubin in poly(I:C)-treated rats; however, the levels were comparable to the normal range. Increased renal expression of bilirubin glucuronide transporters and increased renal clearance have previously been shown to play a compensatory role for reducing the cytotoxic effects of accumulating cholephilic compounds (Tanaka et al., 2002). The effect of poly(I:C) on renal transport in pregnancy awaits elucidation. Furthermore, bilirubin accumulation may be a slower process than changes in accumulation of bile acids. Indeed, a recent study has shown that bile salt levels in the liver were profoundly higher in infected mice much sooner than the onset of direct hyperbilirubinemia (Yang et al., 2009). Studies of duration longer than 24 h may be helpful in clarifying the regulation of bilirubin during maternal infections.

In summary, our results demonstrate that viral mimic, poly(I:C)-induced inflammation significantly down-regulates the expression of several key drug transporters in placentas and livers of pregnant rats. The observed changes are consistent with, but not identical to, those seen with bacterial endotoxin-mediated inflammation, probably because of the involvement of different cytokine pathways in bacterial and viral infections. Moreover, we found that poly(I:C) mediated a significant increase in maternal plasma bile acid levels but did not affect pooled fetal levels of bile acids. However, different substrates are likely to have different outcomes. Because the combined action of the placenta and maternal liver protects the fetus from deleterious effects of endogenous and xenobiotic compounds, it is imperative to further investigate the effect of infections and inflammatory changes on maternal and fetal drug exposure.

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References


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