ATP-Binding Cassette Transporter Expression in Human Placenta as a Function of Pregnancy Condition

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ABSTRACT:

Fetal drug exposure is determined by the type and concentration of placental transporters, and their regulation is central to the development of new treatments and delivery strategies for pregnant women and their fetuses. We tested the expression of several clinically important transporters in the human placenta associated with various pregnancy conditions (i.e., labor, preeclampsia, and preterm labor-inflammation). Placentas were obtained from five groups of women at the time of primary cesarean section: 1) term no labor; 2) term labor; 3) preterm no labor (delivered for severe preeclampsia); 4) preterm labor without inflammation (PTLNI); and 5) preterm labor with inflammation (PTLI). Samples were analyzed by Western blot and immunohistochemistry to identify changes in protein expression. Relative mRNA expression was determined by quantitative real-time polymerase chain reaction. A functional genomic approach was used to identify placental gene expression and elucidate molecular events that underlie the given condition. Placental expression of ATP-binding cassette transporters from women in labor and women with preeclampsia was unaltered. Multidrug resistance protein 1 (MDR1) and breast cancer resistance protein (BCRP) and mRNA expression increased in placentas of women with preterm labor with inflammation. Molecular pathways of genes up-regulated in PTLI samples included cytokine-cytokine receptor interactions and inflammatory response compared with those in the PTLNI group. The mRNA expression of MDR1 and BCRP was correlated with that of interleukin-8, which also increased significantly in PTLI samples. These data suggest that the transfer of drugs across the placenta may be altered in preterm pregnancy conditions associated with inflammation through changes in MDR1 and BCRP.

Introduction

Drug treatment options during pregnancy and lactation are limited because few products have been tested for safety and efficacy in these two patient groups. The placenta is a partially protective barrier that limits fetal exposure to xenobiotics, which is attributed in part to the expression of transporter proteins on placental apical and basal membrane surfaces. Among the most abundant of the apically expressed xenobiotic transporters on the maternal side of the placenta are multidrug resistance protein (MDR) 1 (P-glycoprotein; ABCB1), multidrug resistance-associated protein 2 (MRP2/ABCC2), and breast cancer resistance protein (BCRP/ABCG2), which handle the efflux of xenobiotics and metabolites out of the fetoplacental compartment (Jonker et al., 2000). The localization of MDR3 (ABCB4) and MRP1 (ABCC1) is less clear, but studies suggest that these transporters may be positioned on the basolateral membrane of the placenta where they transport substrates from mother to fetus (Nagashige et al., 2003; Evseeenko et al., 2006). Additional transporters, including BCRP and MRP1, line the fetal capillaries, providing yet another barrier against xenobiotic entry (St-Pierre et al., 2000; Yeboah et al., 2006). Expression of these clinically important transporters is dependent on gestational age. However, drug transporter expression and regulation in placenta of women with pregnancy pathology require further definition. Preterm labor is the leading cause of perinatal morbidity and mortality. Preeclampsia and inflammation, which are often secondary to uterine infection, are well recognized causes of preterm birth and, when diagnosed, frequently result in clinically indicated placental transporters, and their regulation is central to the development of new treatments and delivery strategies for pregnant women and their fetuses. We tested the expression of several clinically important transporters in the human placenta associated with various pregnancy conditions (i.e., labor, preeclampsia, and preterm labor—inflammation). Placentas were obtained from five groups of women at the time of primary cesarean section: 1) term no labor; 2) term labor; 3) preterm no labor (delivered for severe preeclampsia); 4) preterm labor without inflammation (PTLNI); and 5) preterm labor with inflammation (PTLI). Samples were analyzed by Western blot and immunohistochemistry to identify changes in protein expression. Relative mRNA expression was determined by quantitative real-time polymerase chain reaction. A functional genomic approach was used to identify placental gene expression and elucidate molecular events that underlie the given condition. Placental expression of ATP-binding cassette transporters from women in labor and women with preeclampsia was unaltered. Multidrug resistance protein 1 (MDR1) and breast cancer resistance protein (BCRP) and mRNA expression increased in placentas of women with preterm labor with inflammation. Molecular pathways of genes up-regulated in PTLI samples included cytokine-cytokine receptor interactions and inflammatory response compared with those in the PTLNI group. The mRNA expression of MDR1 and BCRP was correlated with that of interleukin-8, which also increased significantly in PTLI samples. These data suggest that the transfer of drugs across the placenta may be altered in preterm pregnancy conditions associated with inflammation through changes in MDR1 and BCRP.
preterm delivery. Treatment for the prevention of preterm birth has thus far been unsuccessful, and the rate of premature birth has increased over the years. We hypothesize that pregnancy conditions associated with preterm birth, such as spontaneous preterm labor, preeclampsia, and preterm labor-inflammation, alter the expression of drug transporters in human placenta. We applied immunohistochemical analysis, Western blot, and quantitative real-time PCR to determine the localization and protein and mRNA expression of transporters in a series of human placentas obtained from women with clinically diagnosed pregnancy conditions. In addition, we applied functional genomic evaluation, an effective approach for obtaining mechanistic understanding of underlying disease through changes in gene expression (Mason et al., 2006), to gain insight into the processes associated with abnormal labor. We postulate that these processes may mediate the observed changes in transporter expression.

The results from these studies provide evidence for altered expression of MDR1 and BCRP during inflammation-associated spontaneous preterm labor. They also support the involvement of cytokine-mediated events as a means to explain the observed increase in MDR1 and BCRP expression. Overall, our data suggest that up-regulation of MDR1 and BCRP could alter drug transfer across the placenta. These results will help predict human fetal drug toxicity and drug delivery and offer new insights into the regulation of placental drug transporters and the impact of various pregnancy conditions on them.

Materials and Methods

Study Design. Placenta were obtained with institutional review board approval and after written consent from five groups of women undergoing primary cesarean section at Yale University: 1) term no labor (TNL); 2) term labor (TL); 3) preterm no labor delivered for severe preeclampsia (PTSPE) [mean gestational age (GA), 30.3 weeks; range, 25.3–36.6 weeks, histological chorioamnionitis, stage 0]; 4) preterm labor unassociated with inflammation (PTLNI) (mean GA, 30.5 weeks; range, 28.0–33.3 weeks, histological chorioamnionitis, stage III); Labor was defined by the presence of regular uterine contractions accompanied by progressive cervical dilation. The diagnosis of intra-amniotic inflammation was based on an amniotic fluid mass restricted score of 3 or 4 plus >100 white blood cells/m³ in the context of a positive amniotic fluid culture in a sample that was obtained by transabdominal amniocentesis (Buhimschi et al., 2005). These tests provide the most accurate tools currently available to maximize the likelihood of sample homogeneity. The mass restricted score provides qualitative information regarding the presence or absence of intra-amniotic inflammation. In brief, the score ranges from 0 to 4, depending on the presence (assigned a value of 1) or absence (assigned a value of 0) of each of four protein biomarkers (Buhimschi et al., 2005). A score of 3 to 4 indicates inflammation, whereas a score of 0 to 2 excludes it. This biomarker pattern is predictive of preterm birth, histological chorioamnionitis, and adverse neonatal outcome. A detailed description of the mass restricted method has been published previously (Buhimschi et al., 2005). Preeclampsia was defined according to established criteria from the American College of Obstetricians and Gynecologists as systolic blood pressure of 140 mm Hg or diastolic blood pressure of 90 mm Hg and proteinuria of at least +1 on dipstick testing, each on two occasions 4 to 6 h apart. In a 24-h urine collection, proteinuria was defined as ≥300 mg of protein. Indications for cesarean delivery in the PTLNI group were related to spontaneous preterm labor. The indication for cesarean delivery in the TNL and TL groups was related to breech presentation and an arrest of cervical dilation at ≥3 cm, respectively. Clinical data were retrieved from the medical records, and statistical analysis of patient demographics was performed using one-way analysis of variance (ANOVA), followed by the Student-Newman-Keuls post hoc test for multiple comparisons.

RNA Isolation and Microarray Preparation. Total RNA isolation and gene profiling of placenta were performed in triplicate for term and preterm samples using the Affymetrix GeneChip Human Genome U133 Plus 2.0 microarray (Affymetrix, Santa Clara, CA) as described previously (Mason et al., 2010).

Microarray Data Processing and Statistical Analysis. The quality of the microarray experiment was assessed as described by Chang et al. (2007) using bioconductor packages for statistical analysis of microarray data. Multidimensional scaling analysis was performed with the signal estimates to assess sample variability. The quality assessment and multidimensional scaling analyses identified and disqualified discordant sample chips. Signal data were obtained using the RMA algorithm. Differential gene expression between the individual pair-wise comparisons was assessed by modified t tests as described previously (Kedzior et al., 2010). The search for genes varying among the conditions was made by combining all the pair-wise comparisons above to construct an F test, which is equivalent to a one-way ANOVA for each gene except that the residual mean squares have been moderated between genes (Smyth, 2004). The p values for the tests provide a way to rank genes in terms of the evidence for differential gene expression to obtain the most likely differentially expressed genes between and among conditions. p ≤ 0.05 and a 1.5-fold threshold were used as a cutoff for gene inclusion in our analysis.

Microarray Data Analysis. DAVID (Huang da et al., 2009), an ontology-based Web tool, was used to evaluate statistical measures of knowledge-based groups of genes from publications and public resources. The biological functions of the genes in the placental groups were examined in DAVID on the basis of information from the Gene Ontology (GO) terms, Kyoto Encyclopedia of Genes and Genomes pathways, and gene descriptions from various public databases. We distinguished genes that were up-regulated and down-regulated (differently expressed genes) and used DAVID to determine Gene Ontology categories that were overrepresented (enriched) with differentially expressed genes. The false discovery rate (FDR) filter identified categories (biological processes, pathways, or molecular functions) that were changed by random chance. The FDR was set at 10%, and GO categories with FDR <10% were considered significantly enriched.

Quantitative Real-Time PCR. Primer sequences for amplifications were chosen on the basis of previously published cDNA sequences (Supplemental Table 1). For normalization of the mRNA data, the endogenous reference gene 18S rRNA was used. All primer sets were tested to ensure efficiency of amplification over a wide range of template concentrations. SYBR Green (Bio-Rad Laboratories, Hercules, CA) was used for amplicon detection. A melt curve was used after amplification to ensure that all samples exhibited a single amplicon. Each sample was assayed in triplicate. The average Ct value (cycle threshold for target or endogenous reference gene amplification) was estimated using the software associated with the iCycler real-time PCR detection system (Bio-Rad Laboratories). Relative changes in mRNA expression of the target genes were analyzed using the ∆∆Ct method (2−∆∆Ct) (Livak and Schmittgen, 2001).

In this method, the average ∆Ct was calculated by subtracting the average Ct value of the endogenous reference gene (18S rRNA) from the average Ct value of the target gene for the condition and control placental groups. Fold changes in mRNA expression of target genes in placenta from the condition groups (TL, PTSPE, PTLNI, and PTLTI) were expressed relative to that of the TNL placental control group. To validate (biological and statistical) the microarray results, we performed qRT-PCR on select genes that were differentially expressed and/or significantly different in comparisons of either PTLSI versus PTLSNI or PTLSI versus TL.

Western Blot Analysis. Human placentas were processed according to methods described previously (Novotna et al., 2004). In brief, placentas were homogenized in buffer containing 250 mM sucrose, 10 mM Tris, 5 mM EDTA, and complete protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN) supplemented with phenylmethylsulfonyl fluoride (1 mM), pH 7.4. Crude membrane fractions were obtained through differential centrifugation. The homogenate was initially centrifuged at 10,000 × g for 10 min at 4°C. The resulting supernatant was then centrifuged at 36,000 × g for 70 min at 4°C. Protein concentration was determined using Bradford (Bio-Rad Laboratories) and protein using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA). Membrane proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked and incubated with primary antibodies against MDR1 and BCRP overnight at 4°C. The membranes were washed and incubated with secondary antibodies, and signals were detected using ECL (Amersham-Pharmacia Biotech).
MDR3 were present in all samples, but their expression was delivery (Camus et al., 2006; Sun et al., 2006). MRP1, MRP2, and dependent on the region of the sample or on cesarean versus vaginal Other laboratories have shown that MDR1 expression is not de-

expression was higher in the placentas of women in the PTLI group was observed in placentas of women with preterm labor (Fig. 1, A and preterm groups (i.e., PTLNI versus PTLI).

Histological grading was based on the four-grade system devised by Salafia et al. (1989). Pathological abnormalities associated with placentas from the PTSPE group consisted of placental infarcts less than 3 cm, fibrin deposition, decidual vascular thrombosis, decidual hem-

Expression and Localization of Placental Drug Transporters. Levels of MDR1, MDR3, MRP1, MRP2, and BCRP were determined from immunoblot analyses. Greater expression of MDR1 and BCRP was observed in placentas of women with preterm labor (Fig. 1, A and B) than in placentas of those with term labor. Furthermore, protein expression was higher in the placentas of women in the PTLI group than in those of women in the PTLNI group (Fig. 1, A and B). Other laboratories have shown that MDR1 expression is not dependent on the region of the sample or on cesarean versus vaginal delivery (Camus et al., 2006; Sun et al., 2006). MRP1, MRP2, and MDR3 were present in all samples, but their expression was variable and did not appear to be dramatically affected by pregnancy condition (Fig. 1, C–E).

The proper cellular localization is essential for transporters to perform their transport function. Immunohistochemical analysis confirmed that the observed changes were due to MDR1 and BCRP (Fig. 2) expression at the membrane of the syncytiotrophoblast cells. BCRP was also localized to fetal blood vessel endothelial cells (Fig. 2). In many instances, the regulation of these transporters occurs at transcription. Given the range of gestational ages in each placental group (condition), we increased the number of placental samples (n = 6–10), after Western blot analysis and immunohistochemical analysis, used for semiquantitative real-time PCR. We found significant increases in MDR1 and BCRP gene expression in the PTLI samples (Fig. 3), which corresponds with their observed protein levels in this condition. There were no changes in MRP1 and MRP2 gene expression among the given conditions. MDR3 mRNA levels were significantly increased in the PTLI group, and there were higher levels in the PTSPE group than in the TL and TNL groups.

Functional Characteristics of Genes Overexpressed in PTLI. PTSDPE compared with PTLNI. We identified 127 genes that were overexpressed (≥1.5; p ≤ 0.05) in PTLI compared with PTLNI. The enrichment of these genes was categorized by GO (pathways, biological processes, and molecular functions). Pathway analysis using the Kyoto Encyclopedia of Genes and Genomes database revealed only one significantly enriched pathway, cytokine–cytokine receptor interaction (FDR 7.7). Significantly up-regulated genes were further categorized by biological processes and molecular functions. We identified seven biological processes that were significantly enriched: 1) response to wounding (FDR 0.6); 2) inflammatory response (FDR 0.8); 3) regulation of cell motion (FDR 3.9); 4) regulation of cell

Results

Clinical Characteristics of Placental Samples. There were no pathological changes in placentas from TNL, TL, and PTLNI groups on the basis of histological evaluation. The samples from the PTLI group were associated with histological stage III chorioamnionitis (full-thickness inflammation of both chorion and amnion). This was complemented by inflammation of the amnion (range, grades 1–3; mode, grade 3), inflammation of the chorion-decidua (range, grades 3–4; mode, grade 3), and funisitis (range, grade 1–4; mode, grade 3). Histological grading was based on the four-grade system devised by Salafia et al. (1989). Pathological abnormalities associated with placentas from the PTSPE group consisted of placental infarcts less than 3 cm, fibrin deposition, decidual vascular thrombosis, decidual hem-

Statistical Analysis. Statistical analysis was done with GraphPad Prism version 4.0; GraphPad Software Inc., San Diego, CA). Quantitative real-time PCR results were reported as fold change in mRNA expression of target genes (mean ± S.E.M.) for each placental group relative to the mRNA expression found in the TNL control placental group. Mean fold changes in mRNA expression in all the groups were compared by ANOVA followed by the post hoc Student-Newman-Keuls multiple comparison test. Pearson coefficient analysis was used to determine the correlation between the fold changes in mRNA expression of the target genes. Statistical significance was set at p < 0.05.

Immunohistochemical Analysis. Immunohistochemical detection of MDR1 and BCRP was performed on frozen sections of placenta from each of the five groups of women (n = 3/group). Placental sections were blocked and incubated overnight at 4°C with the MDR1 and BCRP monoclonal antibodies and dilutions used for Western blots. Biotin-labeled secondary antibodies were visualized using peroxidase-conjugated streptavidin (Vectastain ABC kit; Vector Laboratories, Burlingame, CA) with diaminobenzidine (Sigma-Aldrich) as the substrate. Slides were then counterstained with hematoxylin followed by dehydration in a graded series of ethanol dilutions, cleared by xylene substitute, and mounted with DPX mountant (Sigma-Aldrich). Control incubations did not include primary antibody.

Fig. 1. Immunoblot analysis of protein expression of MDR1 (A), BCRP (B), MRP2 (C), MRP1 (D), and MDR3 (E) in human placentas from women after primary cesarean section during TNL, TL, PTSPE, PTLNI, and PTLI.

Immunohistochemical detection of MDR1 and BCRP was performed on frozen sections of placenta from each of the five groups of women (n = 3/group). Placental sections were blocked and incubated overnight at 4°C with the MDR1 and BCRP monoclonal antibodies and dilutions used for Western blots. Biotin-labeled secondary antibodies were visualized using peroxidase-conjugated streptavidin (Vectastain ABC kit; Vector Laboratories, Burlingame, CA) with diaminobenzidine (Sigma-Aldrich) as the substrate. Slides were then counterstained with hematoxylin followed by dehydration in a graded series of ethanol dilutions, cleared by xylene substitute, and mounted with DPX mountant (Sigma-Aldrich). Control incubations did not include primary antibody.

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proliferation (FDR 5.7); 5) defense response (FDR 6.4); 6) positive regulation of signal transduction (FDR 7.7); and 7) positive regulation of cell motion (FDR 8.0). Three molecular functions were significantly enriched: 1) growth factor binding (FDR 0.1); 2) cytokine binding (1.3); and 3) cytokine receptor activity (FDR 8.4).

PTLI compared with TL. We identified 137 genes that were overexpressed (≥1.5; p ≤ 0.05) in PTLI compared with TL. We found only the focal adhesion pathway (FDR 9.9) to be significantly enriched. Nine biological processes were enriched: 1) female pregnancy (FDR 0.4); 2) tube development (FDR 3.3); 3) ossification (FDR 3.8); 4) positive regulation of kinase activity (FDR 4.3); 5) bone development (FDR 5.1); 6) positive regulation of transferase activity (FDR 5.2); 7) wound healing (FDR 7.08); 8) regulation of locomotion (FDR 7.3); and 9) anion transport (FDR 9.4); two molecular functions were enriched: 1) growth factor binding (FDR 1.95); and 2) actin binding (FDR 5.7).

Functional Characteristics of Genes Underexpressed in PTLI. PTLI compared with PTLNI. We identified 216 genes that were underexpressed (≥1.5; p ≤ 0.05) in PTLI compared with PTLNI. There was only one significantly enriched pathway: extracellular matrix-receptor interaction (FDR 1.7). Ten biological processes were found to be significantly enriched: 1) unsaturated fatty acid metabolic process (FDR 0.6); 2) fatty acid metabolic process (FDR 1.1); 3) branching morphogenesis of a tube (FDR 1.8); 4) morphogenesis of a branching structure (FDR 3.2); 5) eicosanoid metabolic process (FDR 3.9); 6) tube morphogenesis (FDR 6.8); 7) negative regulation of binding (FDR 8.7); 8) lipid biosynthesis process (FDR 8.9); 9) positive regulation of cell adhesion (FDR 9.2); and 10) eicosanoid biosynthesis process (FDR 9.4). Six molecular functions were significantly enriched: 1) lipid binding (FDR 0.4); 2) coenzyme binding (FDR 1.8); 3) cofactor binding (FDR 3.6); 4) actin binding (FDR 6.7); 5) peroxidase activity (FDR 8.1); and 6) oxidoreductase activity, acting on peroxide as acceptor (FDR 8.1).

Biological Validation of Microarray Gene Expression. To verify the microarray results, highly differentially expressed genes including β1 adrenergic receptor (ADRB1), eosinophil major basic protein, also referred to as proteoglycan 2 (MBP or PRG2), stanniocalcin 1 (STC1), and hydroxysteroid (11β-hydroxylase 2 (HSD11B2) were selected and analyzed by qRT-PCR. We confirmed changes in expression (direction and magnitude) of these genes between PTLI and PTLNI (Supplemental Table 2) and PTLI and TL (Supplemental Table 3). Overall, the direction of change in gene expression by qRT-PCR was consistent with the microarray analysis of these four genes. Additional genes encoding human chorionic gonadotropin β polypeptide (βhCG), retinoid X receptor α (RXRa), and GATA binding protein 2 (GATA2) were used to confirm statistical significance of microarray genes.

Changes in the mRNA Expression of Proinflammatory Cytokines in Various Placental Conditions. Previous reports have indicated inverse correlations between MDR1 and proinflammatory cytokines. However, neither TNF-α nor IL-6 mRNA expression was altered, and fold changes in IL-8 mRNA expression were significantly increased (12.1-fold, p < 0.001) in PTLI compared with TNL (Fig. 4). IL-8 mRNA expression in PTLI was greater than that in other conditions including PTSPE, in which the fold change in IL-8 mRNA expression (6.3-fold, p < 0.05) was greater than that of TL but not that of PTL with and without inflammation (Fig. 4). The fold changes in
mRNA expression of IL-8 were correlated with that of MDR1 (Pearson r = 0.50, p < 0.05, respectively) and that of BCRP (Pearson r = 0.65, p < 0.00) among the placental groups.

Discussion

Expression patterns of placental ABC transporters vary with gestational age and medical condition during pregnancy. The general consensus is that MDR1 and BCRP expression decline (Gil et al., 2005; Mathias et al., 2005; Sun et al., 2006; Meyer zu Schwabedissen et al., 2006), whereas MRP2 and MDR3 levels increase with gestational age toward term (Patel et al., 2003; Meyer zu Schwabedissen et al., 2005). These changes may reflect a physiological adaptation to the changing requirements for fetal protection, especially in the preterm period. However, several discrepancies have been observed, particularly in humans. For example, Mathias et al. (2005) reported that BCRP expression in human placenta does not change significantly with gestational age toward term (Patel et al., 2003; Meyer zu Schwabedissen et al., 2005). These changes may reflect a physiological adaptation to the changing requirements for fetal protection, especially in the preterm period. However, several discrepancies have been observed, particularly in humans. For example, Mathias et al. (2005) reported that MDR1 and BCRP expression decline (Gil et al., 2005; Mathias et al., 2005; Sun et al., 2006; Meyer zu Schwabedissen et al., 2006), whereas MRP2 and MDR3 levels increase with gestational age toward term (Patel et al., 2003; Meyer zu Schwabedissen et al., 2005). These changes may reflect a physiological adaptation to the changing requirements for fetal protection, especially in the preterm period. However, several discrepancies have been observed, particularly in humans. For example, Mathias et al. (2005) reported that BCRP expression in human placenta does not change significantly with gestational age toward term (Yeboah et al., 2006) and MDR1 in human placenta (Sun et al., 2006) were not altered by labor at term. Our data further support the fact that MDR1 and BCRP expression does not change with preterm labor. Changes in MRP1, MRP2, and MDR3 expression were less apparent in crude membrane fractions of placental tissue. These preparations differ from isolated syncytiotrophoblasts in purity and may explain potential differences with other results, specifically in the extent of BCRP and MRP1 expression, which is also localized to the fetal capillary endothelial cells. However, immunohistochemical analysis revealed that the cellular localization of MDR1 and BCRP was not altered in the placental groups. Furthermore, mRNA expression appears to parallel that of protein expression. We suspect that the observed differences in protein expression are due to the specific pregnancy condition rather than to variation in experimental design.

Preterm birth is the leading cause of perinatal morbidity and mortality. A large proportion of preterm births are associated with preeclampsia and inflammation, often secondary to infection. It is increasingly clear that inflammation (outside of that associated with pregnancy) affects the expression of drug transporters (Petrovic et al., 2007). We found that both MDR1 and BCRP (protein and mRNA expression) are highest in placentas from women with inflammation (Figs. 1, A and B, and 3, A and B). Given their high white blood cell...
counts, it is probable that inflammation (i.e., stage III chorioamnionitis) is a response to uterine infection. These data represent the first evidence of direct infection-mediated transporter regulation.

Our findings differ from prior literature reports noting transporter down-regulation during inflammation caused by inflammatory cytokines such as TNF-α, IL-6, and endotoxin (i.e., LPS) in rats (Sukhai et al., 2001; Chen et al., 2005; Wang et al., 2005) and human primary placental cells (Evseenko et al., 2007). We offer several possible explanations for these differences: the impact of LPS-induced inflammation on drug transporters has yet to be evaluated at different gestational stages, and previous reports have indicated that preterm placentas respond differently to LPS than those at term, specifically in their patterns of cytokine release (Holcberg et al., 2007). More importantly, common clinical infections of the reproductive compartment are associated with microorganisms that lack LPS, such as Ureaplasma species, Mycoplasma hominis, and group B Streptococcus. It is evident that different pathogens or pathogen components elicit diverse patterns of gene expression and cytokine release (Flad et al., 1993; Ueyama et al., 2005). For example, IL-8 was significantly elevated in amniotic fluid and umbilical cord blood in cases of intrauterine Ureaplasma infection, which was not observed with other pathogens (Witt et al., 2005). Taken together, these results indicate that stimulation of alternative cytokines or inflammatory mediators could have contrasting affects on ABC transporters. Thus, observed differences in transporter regulation among various experimental models are not surprising. This is evident in cases of patients with inflammation from rheumatoid arthritis in whom an increase, rather than a decrease, in MDR1 expression is observed (Llorente et al., 2000). It is clear that the impact of inflammation on drug transporters in the human placenta is still in a nascent stage. The development of models that more closely mimic the human pathological pregnancy condition will expound differences in transporter regulation, including the need to evaluate various inflammatory pathogens and or stimuli during pregnancy.

Hence, we adopted a functional genomic approach to identify potential mechanisms driving changes in gene expression during PTI. We hypothesized that underlying inflammatory events may account for the observed MDR1 and BCRP regulation. Because the PTI group is defined, in part, by labor, it was logical to compare this placental group with those also associated with labor, specifically PTLN and TL. In general, functional pathways and biological processes associated with pregnancy and development were found to be enriched (overrepresented) with genes overexpressed in PTI compared with TL. Of interest, these events appeared to be similar in comparisons of genes up-regulated in PTLN compared with TL (supplemental data). When we compared PTLI with PTLN, we found that genes were up-regulated in processes associated with inflammation and cellular regulation, in particular, the cytokine-cytokine receptor interaction pathway, as were molecular functions related to cytokine activity. These results provide biological relevance for the given PTLI condition and further suggest that proinflammatory cytokines may be involved in the pathways regulating MDR1 and BCRP. Thus, we evaluated the correlation between expression of well recognized proinflammatory cytokines, IL-6, IL-8, and TNF-α and MDR1 and BCRP.

IL-8 is a potent chemotactic agent and activates neutrophils, potentiating the host defense mechanism against inflammation. It is thought to be constitutively produced by the human placenta (Shimoya et al., 1992) independent of preterm versus term delivery (Keelan et al., 1999). IL-8 is increased in placental tissue during...
choioamnionitis (Lockwood et al., 2006) as well as in amniotic fluid and cord blood from women with intrauterine infection. We found a significant fold increase in IL-8 mRNA expression in placenta in PTLI, whereas there were no differences in placenta in preterm versus term pregnancy as demonstrated in comparisons between PTLNI and TL and TNL (Fig. 4). These results are consistent with the literature. Fold changes in IL-8 mRNA expression were correlated with that of MDR1 and that of BCRP. On the basis of the aforementioned association between IL-8 and inflammation-infection, these data support altered expression of MDR1 and BCRP in placenta of women with preterm labor and inflammation. Of interest, there were no changes in mRNA expression of other proinflammatory cytokines, TNF-α and IL-6. However, changes in these cytokines may be more apparent in the amniotic fluid or the maternal or fetal serum.

We observed elevated placental maternal mRNA expression of IL-8 in women with PTSP compared with women with term labor (TNL and TL). These results are consistent with reports of increased IL-8 production in trophoblasts (Bowen et al., 2005) and elevated IL-8 levels in maternal and umbilical cord serum as well as amniotic fluid of preeclamptic women (Nakabayashi et al., 1998; Laskowska et al., 2007). In contrast, Wang et al. (1999) found a decrease in placental IL-8 production in preeclampsia. Additional experiments may be required to determine the association of preeclampsia and cytokine-specific production.

In this study, we did not detect significant changes in protein or mRNA expression of the multidrug-associated proteins, MRP1 and MRP2. LPS and proinflammatory cytokines have been shown to down-regulate MRP2 expression in the liver of rodents (Teng and Piquette-Miller, 2008); however, there are currently no data to support inflammatory-induced changes in MRP2 and MRP1 expression in humans and in placental tissue. Although MDR3 has generally been considered a liver-specific transporter, MDR3 expression in human term and preterm placentas has been described previously (Patel et al., 2003); however, its physiologic function in syncytiotrophoblasts remains speculative. We observed that MDR3 levels were not altered to the same extent as its mRNA expression. Others have also indicated discrepancies in MDR3 and its mRNA expression in trophoblasts, which may be attributed, in part, to translational regulation (Evseenko et al., 2006).

In the present study, we found that MDR1 and BCRP are significantly regulated in human placenta. Prior studies have shown that MDR1 and BCRP are coregulated in various tissue barriers to enhance tissue protection from xenobiotics. For example, de Vries et al. (2007) showed that these two transporters act in concert to limit the penetration of toptocan at the blood-brain barrier. Like the blood-brain barrier, the placenta protects against harmful toxic substances and restricts the entry of therapeutic agents. Therefore, changes in placental expression of these transporters could have a profound impact on drug efficacy or toxicity. We further demonstrated that both MDR1 and BCRP expression increase in association with underlying inflammation. Up-regulation of MDR1 and BCRP in placenta during preterm labor and/or delivery could significantly impair therapeutic intervention. For example, MDR1 and BCRP transport a variety of drugs including drugs necessary for fetal therapy. BCRP/BCrP significantly limits the fetal level of nitrofurantoin, an antibiotic commonly used to treat urinary tract infections during pregnancy (Zhang et al., 2007), whereas MDR1/Mdrla/b transports antibiotics such as azithromycin, erythromycin, clarithromycin, levofloxacin, and rifampin (Therauf and Fromm, 2006), agents currently used to prevent maternal-fetal infections. MDR1 may also limit the transplacental transfer of protease inhibitors such as nelfinavir, ritonavir, saquinavir, and lopinavir, which are used in human immunodeficiency virus-infected women to prevent transmission to the fetus. At the present time, perinatal drug therapy in an inflamed and/or infected maternal-fetal milieu is secondary to clinical premature fetal delivery. Further studies will be needed to demonstrate that placental MDR1 and BCRP expression during preterm inflammatory conditions directly correlates with drug exposure and outcome. A variety of placental transporters localize to the maternal interface of the placenta, the fetal membrane surface, or both. Additional studies should be focused on other important placental transporter proteins and their regulation under various pregnancy conditions.

Authorship Contributions

Participated in research design: Mason, Weiner, and Swaan.

Conducted experiments: Mason and Dong.

Contributed new reagents or analytic tools: I.A. Buhimschi and C.S. Buhimschi.

Performed data analysis: Mason and Swaan.

Wrote or contributed to the writing of the manuscript: Mason, Weiner, and Swaan.

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