Prominent Expression of Xenobiotic Efflux Transporters in Mouse Extraembryonic Fetal Membranes Compared with Placenta

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ABSTRACT:

Fetal exposure to xenobiotics can be restricted by transporters at the interface between maternal and fetal circulation. Previous work identified transporters in the placenta; however, less is known about the presence of these transporters in the fetal membranes (i.e., yolk sac and amniotic membranes). The purpose of this study was to quantify mRNA and protein expression of xenobiotic transporters in mouse placenta and fetal membranes during mid to late gestation. Concepti (placenta and fetal membranes, gestation day 11) or placenta and fetal membranes (gestation days 14 and 17) were collected from pregnant mice and analyzed for expression of multidrug resistance-associated proteins (Mrps), multidrug resistance proteins (Mdrs), multidrug and toxin extrusion proteins (Mates), breast cancer resistance protein (Bcrp), and organic anion-transporting polypeptides (Oatps). Maternal liver and kidneys were also collected at day 14 for mRNA and immunohistochemical analysis. mRNA expression of Mrp, Mdr, Bcrp, Mate-1, and Oatp isoforms was detected at day 11. The uptake carriers Oatp2a1, 3a1, 4a1, and 5a1 showed placenta-predominant expression. At days 14 and 17, fetal membranes expressed higher mRNA levels of the efflux transporters Mrp2 (7-fold), Mrp4 (5-fold), Mrp5 (3-fold), Mrp6 (12-fold), Bcrp (2-fold), and Mate-1 (7-fold) than placenta. Western blot analysis of Mrp2, Mrp4, Mrp6, and Bcrp confirmed higher expression in fetal membranes. Immunostaining revealed apical (Mrp2 and Bcrp) and basolateral (Mrp4, 5, and 6) cellular localization in epithelial cells of the yolk sac. In conclusion, xenobiotic transporters in the fetal membranes may provide an additional route to protect the fetus against endogenous chemicals and xenobiotics.

Bidirectional exchange of chemicals into and away from the fetus is critical for normal in utero development. Nutrients and endogenous chemicals are transferred from the maternal circulation to the fetus, thereby providing building blocks for organogenesis. Likewise, metabolic by-products produced by the fetus are transferred to the mother. Chemical and nutrient transport between fetus and mother occurs through the placenta and fetal membranes. In rodents, the fetal membranes comprise an inverted yolk sac and amniotic membrane that extend from the placenta and enclose the fetus. In addition to providing nutrition, immunologic defense, and gas exchange, the placenta and fetal membranes likely represent physical barriers that prevent fetal exposure to potentially harmful xenobiotics by limiting passage, enhancing removal, or both.

Membrane transport proteins import and efflux chemicals into and from cells, respectively. Consequently, transporters play key roles in the clearance of drugs and endobiotics from the body as well as protection of sanctuary organs such as the testes and brain. Active transport of substrates into and from tissues depends on the orientation and localization of transporters in the plasma membrane of polarized cells. In the liver and kidney, members of the organic anion-transporting polypeptide (Oatp) family participate in the uptake of chemicals into epithelial cells. In contrast, efflux transporters remove chemicals and their metabolites from hepatocytes and renal tubule cells. Families of efflux transporters include the multidrug resistance-associated proteins (Mrp), multidrug resistance proteins (Mdrs) P-glycoprotein (Pgp), multidrug and toxin extrusion proteins (Mate), and the breast cancer resistance protein (Bcrp). Localization of these transporters to either the apical or basolateral membranes of hepatocytes and renal tubule cells determines the direction of chemical exchange. Although transporter isoforms tend to be expressed at the same side of polarized epithelia within different tissues, there are instances in which this does not occur. For example, Mrp4 protein is expressed on the basolateral membrane of hepatocytes, but on the apical face of proximal tubule cells (van Aubel et al., 2002; Aleksunes et al., 2006). Because of such discrepancies, it is important to identify the localization of transporters within various tissues.

Because of the critical role of the placenta in fetal development and protection, researchers have characterized mRNA and protein levels of transporters in rodent and human placenta. The uptake transporters, OATP2B1 (OATP-B), OATP3A1 (OATP-D), and OATP4A1 (OATP-E) are expressed in human placenta (St-Pierre et al., 2002; Sato et al., 2003; Ugele et al., 2003). Similarly, rodent Oatp1a1, 1a5, 1b2, 2a1, 2b1, and 4a1 homologs have been detected in rat placenta.

ABBREVIATIONS: Oatp/OATP, rodent/human organic anion transporting polypeptide; Mrp/MRP, rodent/human multidrug resistance-associated protein; Mdr/MDR, rodent/human multidrug resistance protein; Pgp, PGP, rodent/human P-glycoprotein; Mate/MATE, rodent/human multidrug and toxin extrusion proteins; Bcrp/BCRP, breast cancer resistance protein; PBS, phosphate-buffered saline; bDNA, branched DNA; PBS-Tx, phosphate-buffered saline with 0.1% Triton X; DAPI, 4',6-diamidino-2-phenylindole.

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obtained during mid to late gestation (Leazer and Klaassen, 2003; St-Pierre et al., 2004).

A number of efflux transporters, including MRPI–3 and 5, BCRP, and MDR1/PGP, are present in syncytiotrophoblasts and, to a lesser extent, in endothelial cells of capillaries within human placenta (St-Pierre et al., 2000; Nagashige et al., 2003; Pascolo et al., 2003; Kolwankar et al., 2005; Mathias et al., 2005). There are notable changes in expression of some placental efflux transporters during gestation. Bcrp mRNA and protein levels peak in rodent placenta during the middle of gestation and decline during later stages (Yasuda et al., 2005; Wang et al., 2006; Kalabis et al., 2007). A recent publication demonstrated MRPI, 2, 5, and BCRP protein expression in term human amnion membranes obtained after caesarean section (Aye et al., 2007). Bcrp and Mrp1 proteins have also been detected on the apical membrane of syncytiotrophoblasts and endodermal epithelia of rodent yolk sac (St-Pierre et al., 2004; Wang et al., 2006; Kalabis et al., 2007). Pgp protein is localized to the luminal surface of trophoblasts (facing maternal circulation) (Novotna et al., 2004; Kalabis et al., 2005). Little is known about the expression of Mate-1 and Mrp4–6 in placenta and fetal membranes. Furthermore, the relative expression of transporters in the fetal membranes compared with that in placenta has been largely undetermined.

Therefore, the purpose of this study was to investigate the expression of uptake and efflux transporters in mouse placenta and fetal membranes during gestation (days 11, 14, and 17). The average gestation period in mice is 19 to 21 days. Implantation occurs during early gestation (days 0–5) followed by gastrulation (days 5–10) and organogenesis (days 10–14). Late gestation (days 14–19) includes late organogenesis, fetal growth, and development. Identification and characterization of transporter proteins in placenta and fetal membranes will enable researchers to determine the types of substrates to which a fetus is likely to be exposed during different periods of gestation.

Materials and Methods

Animals. Female C57BL/6 mice (Charles River Laboratories, Inc., Wilmington, MA) were mated overnight (beginning at 5 PM) and males were removed the following morning at 7 AM. This was designated as gestation day 0 (average gestation period of 19 days). Average litter size for C57BL/6 mice is seven in our facility. For RNA and protein analysis, the whole conceptus was obtained along with the placenta and fetal membranes and a portion of each tissue was carefully dissected and separated. Maternal livers and kidneys were accurately separated. On days 14 and 17, the placenta and fetal membranes were frozen in liquid nitrogen. Animals received humane care as outlined in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996). Studies were approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee.

RNA Isolation. Fetal membranes from three concepti were pooled before RNA isolation and were designated a single sample (nine fetal membranes yielded three samples). Total RNA was isolated using RNABee reagent (Tel-Test Inc., Friendswood, TX) according to the manufacturer’s protocol. The concentration of total RNA was quantified spectrophotometrically at 260 nm.

Branchoned DNA Signal Amplification Assay. The mRNA expressions of mouse Outila5, 2a1, 2b1, 3a1, 4a1, and 5a1, Mrp1–6, Mdr1a and 1b, Mate-1, and Bcrp were quantified using the bDNA signal amplification assay (Quantigene, High Volume bDNA Signal Amplification Kit 1.0; Panomics, Fremont, CA). Multiple oligonucleotide probe sets (containing capture, label, and blocker probes) specific to mouse mRNA transcripts were designed using ProbeDesigner software (version 1.0; Bayer Corp., Diagnostics Div., Tarrytown, NY). Specific mouse probe sets were described previously (Cheng et al., 2005; Maher et al., 2005; Tanaka et al., 2005; Cheng and Klaassen, 2006).

Western Blotting. Placenta and fetal membranes were homogenized separately in sucrose-Tris buffer (0.25 M sucrose and 10 mM Tris-HCl, pH 7.4) using a Potter-Elvehjem homogenizer. Protein concentrations were determined using Pierce BCA protein assay reagents according to the manufacturer’s recommendations (Pierce Biotechnology, Rockford, IL). Homogenates (40 μg of protein/lane) were loaded and separated on 8% SDS-polyacrylamide gels. Proteins were transferred overnight at 4°C to polyvinylidene difluoride membranes. Membranes were blocked for 1 h in blocking buffer (2% nonfat dry milk with 0.5% Tween 20). All primary and secondary antibodies were diluted in blocking buffer. Primary antibody dilutions are as follows: Mrp1 (Mrp1, 1:2000), Mrp2 (M3II-5, 1:600), Mrp3 (M3II-2, 1:1000), Mrp4 (M4I-10, 1:1000), Mrp5 (M5I-10, 1:1000), Mrp6 (M6II-68, 1:1000), Bcrp (BX65-53, 1:5000), Pgp (C219, 1:1000), and β-actin (ab8227, 1:2000). Mrp and Bcrp antibodies were provided by George Scheffer, VU Medical Center (Amsterdam, the Netherlands). The β-actin antibody was purchased from Abcam Inc. (Cambridge, MA). The C219 antibody was purchased from Signet Laboratories ( Dedham, MA). Blots were subsequently incubated with a species-appropriate horseradish peroxidase-conjugated secondary antibody for 1 h (Sigma-Aldrich, St. Louis, MO). Protein-antibody complexes were detected using an ECL chemiluminescent kit (Pierce Biotechnology) and exposed to medical X-ray film (Denville Scientific, Metuchen, NJ). The intensity of protein bands was quantified using Quantity One software (Bio-Rad, Hercules, CA).

Immunofluorescence Analysis. Staining for Mrp2, Mrp4, Mrp5, Mrp6, and Bcrp was performed as described previously (Alekseenus et al., 2006). Of note, the M3II-5 antibody does not detect mouse Mrp2 protein by immunochemical staining. In turn, the Mrp2 antibody from Bruno Steiger (University Hospital Zurich, Zurich, Switzerland) was used for immunohistochemical analysis. Cyanoseseions (5 μm) were thaw-mounted onto Superfrost glass slides (Thermo Fisher Scientific) and stored at ~80°C with desiccant until use. In brief, cyanoseseions were blocked with 5% goat serum in phosphate-buffered saline containing 0.1% Triton X (PBS-Tx) for 1 h and then incubated with primary antibody diluted 1:100 in 5% goat serum/PBS-Tx for 2 h at room temperature. Sections were subsequently washed in PBS-Tx and incubated for 1 h with goat anti-rat Alexa 488 IgG for detection of Mrp4, Mrp5, Mrp6, and Bcrp, whereas goat anti-rabbit Alexa 488 IgG was used for detection of Mrp2 (Invitrogen, Carlsbad, CA). Secondary antibodies were diluted 1:200 in 5% goat serum/PBS-Tx. Rhodamine-phalloidin (1:200; Invitrogen) was added to secondary antibody incubations for detection of actin cytoskeleton. For double staining, anti-rabbit Alexa 594 F(ab’2) fragment was used for detection of Mrp2 (Invitrogen). The sections were air-dried and mounted in Prolong Gold with 4’,6-diamidino-2-phenylindole (DAPI) (Invitrogen). Images were captured on an Olympus BX41 fluorescent microscope with a DP70 camera and DP controller software. Each fluorescent channel was acquired sequentially and then merged to create the final image. Images were cropped, and brightness and contrast were adjusted the same for each slide in Adobe Photoshop CS2 (Adobe Systems, San Jose, CA). Day 14 fetal membranes were used for immunofluorescent staining because protein expression of efflux transporters as determined by Western blot was highest at this time point. All sections were both stained and imaged under uniform conditions for each antibody. Negative controls without antibody were included in the analysis (data not shown).

Statistical Analysis. Quantitative results were expressed as mean ± S.E. For bDNA analysis, data are presented as relative light units per 4 μg of total RNA. Immunoblot data are presented as trace density (optical density × millimeters). Statistical analysis was not performed for tissue distribution analysis (Figs. 1 and 2). Quantitative results in Figs. 3 to 5 were analyzed by one-way analysis of variance followed by Tukey’s multiple range test (p < 0.05) using GraphPad Prism software (version 4; GraphPad Software Inc., San Diego, CA). Two-way analysis was subsequently used to confirm the influence of tissue (placenta and fetal membranes) and time (gestation days 14 and 17) on transporter expression. Day 11 placenta/fetal membranes were excluded from statistical analysis because the two tissues could not be accurately dissected.
Results

Tissue Expression of Uptake Transporters. Placenta, fetal membranes, and maternal liver and kidney at gestation day 14 were analyzed for mRNA expression of six Oatp carriers to determine tissue distribution and relative expression between maternal and embryonic tissues (Fig. 1). mRNA levels of Oatp1a5 were similar among all four tissues, with a tendency for higher expression in maternal kidney. Expression of Oatp2a1, 4a1, and 5a1 mRNA was seen predominantly in mouse placenta, with lower levels in the other tissues analyzed. Oatp3a1 was also detected in mouse placenta; however, its expression was half that in maternal kidneys. In contrast, Oatp2b1 mRNA was very low in placenta and fetal membranes, with markedly higher levels in maternal liver and kidney. Expression of Oatp1a1, 1a4, and 1b2 mRNA in placenta and fetal membranes was less than 1% of that in adult mouse liver (data not shown).

Tissue Expression of Efflux Transporters. Analysis of Mrp1–6, Mdr1a and 1b, Bcrp, and Mate-1 mRNA expression in placenta, fetal membranes, and maternal liver and kidney at gestation day 14 revealed relatively higher expression of efflux transporters (Mrp2, Mrp4, Mrp5, Mrp6, Bcrp, and Mate-1) in fetal membranes than in placenta (Fig. 2). These transporters were also highly expressed in maternal liver (Mrp2, Mrp6, and Mate-1) and kidney (Mrp2, Mrp4, Mrp6, Bcrp, and Mate-1). Hepatic and renal Mrp5 mRNA levels were similar to expression in mouse placenta. Mrp1, Mdr1a, and Mdr1b mRNA were higher in the placenta than in liver, kidney (except Mrp1), and fetal membranes. Very low levels of Mrp3 were detected in mouse placenta and fetal membranes compared with those in liver and kidney.

Gestational Expression of Uptake Transporters in Placenta and Fetal Membranes. Because levels of a number of Oatp transporters were higher in mouse placenta compared to fetal membranes, a time course study was designed to quantify expression of Oatps from gestation days 11 to 17 in these two tissue compartments (Fig. 3). Because placenta and fetal membranes could not be accurately separated at day 11, mRNA values at this time point reflect their combined expression. Oatp2a1, 2b1, 3a1, 4a1, 5a1, and 5a1 are lower degree, Oatp1a5 mRNA were detected in mouse placenta/fetal membranes at gestation day 11. Similar to Fig. 1, gene expression of Oatp2a1, 3a1, 4a1, and 5a1 was higher in placenta than in fetal membranes. Levels of Oatp2a1 and 2b1 mRNA were similar between fetal membranes and placenta over the time course. Expression of Oatp2a1 mRNA in placenta increased 1.6-fold from day 14 to 17, whereas the level of Oatp5a1 decreased 46%. Two-way ANOVA revealed significant effects of tissue type (i.e., placenta) on the expression of Oatp3a1 and 4a1 and significant effects of tissue type and gestational day on the expression of Oatp2a1 and 5a1.
Relative placenta/membrane expression demonstrates the following rank order at gestation day 14: Oatp4a1 (24-fold), 5a1 (21-fold), 3a1 (6-fold), 2a1 (3-fold), 2b1 (2-fold), and 1a5 (2-fold). A similar rank order was observed at gestation day 17: Oatp4a1 (23-fold), 3a1 (8-fold), 5a1 (6-fold), 1a5 (3-fold), 2a1 (3-fold), and 2b1 (80% of fetal membranes).

**Gestational Expression of Efflux Transporters in Placenta and Fetal Membranes.** Mrp1–6, Mdr1a, 1b, Bcrp, and Mate-1. The data are presented as mean relative light units (RLU) per 4 µg of total RNA ± S.E. (n = 3).
were detected in placenta and fetal membranes at gestation day 11. Similar to Fig. 2, the gestational time course study revealed higher expression of Mrp2, Mrp4 (day 14 only), Mrp5, and Mrp6 mRNA in fetal membranes than in placenta at days 14 and 17 (Fig. 4). Interestingly, Mrp4 expression in fetal membranes decreased from gestation day 14 to 17. Mrp1 mRNA was lower in fetal membranes with expression at 16 and 28% of placental levels at days 14 and 17, respectively. Two-way ANOVA revealed significant effects of tissue type (i.e., fetal membranes) on the expression of Mrp1, 2, 5, and 6 and significant effects of tissue type and gestation day on the expression of Mrp4.

At day 14, expressions of Mate-1 and Bcrp mRNA were 7- and 2-fold higher in fetal membranes than in placenta, respectively (Fig. 4). By day 17, Bcrp expression in fetal membranes decreased, whereas Mate-1 levels remained unchanged. Mdr1a mRNA was lower in fetal membranes than in placenta at day 17. In contrast to Mdr1a, levels of Mdr1b were similar in placenta and fetal membranes at days 14 and 17. Two-way ANOVA revealed significant effects of tissue type (i.e., fetal membranes/placenta) on the expression of Mdr1a and Mate-1 and significant effects of tissue type and gestation day on the expression of Bcrp.

Relative membrane/placenta expression demonstrates the following rank order at gestation day 14: Mrp6 (12-fold), Mrp2 (7-fold), Mate-1 (7-fold), Mrp4 (5-fold), Mrp5 (3-fold), Bcrp (2-fold), Mrp3 (no difference), Mdr1a (50% of placenta), Mdr1b (33% of placenta), and Mrp1 (28% of placenta). A similar rank order was observed at gestation day 17: Mrp2 (8-fold), Mate-1 (6-fold), Mrp6 (5-fold), Mrp5 (3-fold), Mrp4 (2-fold), Bcrp (2-fold), Mdr1b (no difference), Mdr1a (41% of placenta), and Mrp1 (16% of placenta).

**Protein Expression of Efflux Transporters in Placenta and Fetal Membranes.** Western blot analysis was performed on placenta and fetal membrane homogenates at days 14 and 17 (Fig. 5). Analysis was limited to Mrp1–6, Bcrp, and Pgp. Protein analysis of other transporters whose mRNA expression had been assessed by bDNA was not performed because there are no specific antibodies commercially available to detect these proteins in mice (e.g., Oatps and Mate-1). Individual blots are shown in Fig. 5A. Expression of Mrp1 protein increased 26-fold in mouse placenta from gestation days 14 to 17. Little Mrp1 protein was detected in fetal membranes. In contrast to Mrp1, other efflux transporters, including Mrp2, Mrp4, Mrp6, and Bcrp, demonstrated fetal membrane-predominant expression patterns. Mrp2 protein expressions in fetal membranes were 5- and 2-fold higher than that in the placenta at gestation days 14 and 17, respectively. Similarly, Mrp4 protein was 136-fold higher in fetal membranes than that in placenta at gestation day 14; however, Mrp4 levels
FIG. 4. Gestational expression of efflux Mrp, Mdr, Bcrp, and Mate transporters in placenta and fetal membranes. Total RNA was isolated from concepti (day 11) and placenta and fetal membranes (days 14 and 17). RNA was analyzed by bDNA assay for expression of Mrp1–6, Mdr1a, Mdr1b, Mate-1, and Bcrp. The data are presented as mean relative light units (RLU) per 4 μg of total RNA ± S.E. (n = 3–5), *, statistical differences (p < 0.05) between placenta and fetal membranes; †, statistical differences (p < 0.05) compared with day 14.
Fig. 5. Protein expression of efflux transporters in placenta and fetal membranes. Western immunoblots were performed using homogenates (40 μg of protein/well) from placenta and fetal membranes at gestation days 14 and 17. The data are presented as individual blots (A) and as mean trace density (optical density × millimeter) ± S.E (n = 3) (B) for Mrp1, Mrp2, Mrp4, Mrp5, Mrp6, Bcrp, and Pgp. Blots were probed for β-actin staining to confirm equal protein loading. Because of differences in relative expression of β-actin between placenta and fetal membranes, transporter Western blot data were not normalized to β-actin. *, statistical differences (p < 0.05) between placenta and fetal membranes. †, statistical differences (p < 0.05) compared with day 14.
were markedly reduced by day 17. Mrp5 was detected in placenta and membranes at day 14, with reduced expression in both tissues by day 17. Mrp6 protein was not detected in placenta but was detected in fetal membranes and decreased from day 14 to 17. Bcrp protein levels were 6- and 3-fold higher in fetal membranes than that in placenta at days 14 and 17, although the relative difference was not statistically significant at day 17. Placenta and fetal membranes had similar expressions of Pgp, with reduced levels seen at day 17. Of note, the Pgp antibody (C219) does not discriminate the various Mdr isoforms (Vos et al., 1998). Mrp3 protein could not be detected in mouse placenta or fetal membranes (data not shown). It should be noted that placental expression of β-actin (loading control) is 2-fold higher than that in fetal membranes at both time points.

**Immunofluorescent Detection of Efflux Transporters in Fetal Membranes.** Indirect immunofluorescent staining was performed on cryosections from gestation day 14 fetal membranes to determine patterns of cellular distribution and localization of transporters. Because hepatic and renal expression of efflux transporters is known, maternal liver and kidneys were also stained to confirm antibody staining patterns. Immunofluorescent staining of Mrp4, Mrp5, and Mrp6 (green) demonstrated staining of epithelial cells (endoderm-derived) in the visceral yolk sac at gestation day 14 (Fig. 6, A, F, and K, respectively). Little staining was observed in the amnion membrane (data not shown). Higher magnification shows basolateral localization of these three efflux transporters (Fig. 6, B, G, and L). To differentiate Mrp4–6 staining with the entire plasma membrane, tissue sections were also stained with rhodamine-phalloidin which detects actin (red) (Fig. 6, C, H, and M). Comparison of Mrp4–6 and actin images demonstrates little transporter staining at the apical surface of the yolk sac.

As previously reported, Mrp4 protein expression in maternal kidney (Fig. 6E) was localized to the apical brush-border membrane (Leggas et al., 2004). Little hepatic Mrp4 protein staining was observed (Fig. 6D). Similarly, Mrp5 was not detected in maternal liver and kidney sections (Fig. 6, I and J). Staining for Mrp5 protein revealed basolateral localization in maternal hepatocytes (Fig. 6N) and proximal tubules (Fig. 6O).

Bcrp and Mrp2 proteins (green) are expressed at gestation day 14 and exhibit apical cellular localization in epithelial cells (endoderm-derived) of the visceral yolk sac (Fig. 7, A and F, respectively). Staining of the actin cytoskeleton (red) in Fig. 7, C and H, confirms the fact that Bcrp and Mrp2 staining is not localized to the lateral or basal membranes (Fig. 7, B and G). Little staining was observed in the amniotic membrane (data not shown). Similar to fetal membranes, Bcrp and Mrp2 proteins are localized to apical membranes of maternal hepatocytes (canalicular membrane) (Fig. 7, D and I, respectively) and proximal tubule cells (brush-border membrane) (Fig. 7, E and J, respectively).

Double immunostaining directed against Bcrp (green, Fig. 8A) and Mrp2 (red, Fig. 8B) confirmed that both efflux transporters are localized to the apical surface of the yolk sac (Fig. 8C). This is in contrast to double staining of Mrp2 (red, Fig. 8E) and Mrp4 (green, Fig. 8D), in which the two proteins demonstrate distinct localization to the apical and basolateral membranes, respectively (Fig. 8F). Double staining of Mrp2 with either Mrp5 or Mrp6 did not reveal overlap in localization (data not shown).

### Discussion

The present study characterizes the expression of uptake and efflux transporters in mouse placenta and fetal membranes during gestation. The relative mRNA expression of transporters between placenta, fetal membranes, and maternal liver and kidney were isoform-dependent. Data generated in the current study suggest that placental transporter expression in mice parallels patterns published for humans. Detection of Mrp1, Mrp5, Pgp, and Bcrp proteins in mouse placenta was similar to that noted previously in human placenta (Nagashige et al., 2003; Mathias et al., 2005; Meyer zu Schwabedissen et al., 2005a). Human and mouse placenta express the same uptake transporter.
isoforms including OATP2B1/2b1, 3A1/3a1, and 4A1/4a1 (St-Pierre et al., 2002; Sato et al., 2003; Ugele et al., 2003). In addition to similarities in placental expression, MRP1/Mrp1, MRP2, MRP5, and BCRP/Bcrp proteins have been detected in human amnion and rodent yolk sac (St-Pierre et al., 2004; Aye et al., 2007; Kalabis et al., 2007). Whereas rodent and human placenta are both hemochorial, the physical structure of the placental unit differs between the two species (Carter, 2007). Invasion of trophoblasts into uterine arteries is more extensive in human placenta, with a single layer of syncytiotrophoblasts as the site of exchange. In contrast, rodent placenta comprises three trophoblast layers. In addition, human yolk sac floats free in exocoelom during the first trimester, whereas rodents exhibit an inverted visceral yolk sac that functions until term. Despite these structural differences, the rodent remains a useful model for assessing pharmacokinetics within the embryo due to the general similarities in transporter expression noted above. Use of knockout mice is gaining popularity for determining the role of a particular transporter in the accumulation of xenobiotics and teratogens within the fetus. The antibiotic nitrofurantoin accumulates within fetuses of Bcrp-null mice, resulting in a 4-fold increase in the fetal/maternal plasma area under the curve ratio, compared with that in wild-type mice (Zhang et al., 2007). Mdr1a/1b double null mice exhibit increased fetal/maternal plasma levels of digoxin, saquinavir, and paclitaxel, confirming a role for Pgp in limiting transplacental passage of drugs (Smit et al., 1999). In addition, CF-1 mice with a natural mutation in Pgp have increased accumulation of the L-652,280 isomer of the pesticide, avermectin, in the fetal compartment, resulting in an elevated incidence of cleft palate malformations (Lankas et al., 1998). Knowledge generated in the present study will enable the design of additional in vivo disposition and toxicology studies for transporters (i.e., Mrp2 and Mrp6) not previously investigated in the placenta and fetal membranes.

The fetus is particularly sensitive to xenobiotic toxicity during early gestational periods. Limited availability of tissue in pregnant mice makes it difficult to quantify transporter expression during the first half of gestation (days 1–10). A number of studies using human preterm placenta suggest that transporter expression declines with gestation. Levels of MRP2, MRP5, PGP, and BCRP are higher in

FIG. 7. Immunofluorescent detection of Bcrp and Mrp2 transporters in fetal membranes. Indirect immunofluorescence against Bcrp, Mrp2 (green), and actin cytoskeleton (red) was performed on day 14 fetal membrane cryosections. Images are shown at 40× (A and F) magnification. Cropped images were enlarged and provided as insets (B and G). Actin staining is shown at high magnification (C and H). Sections were mounted in Prolong Gold containing DAPI for nuclear staining (blue). Maternal liver (D and I) and kidney (E and J) cryosections from gestation day 14 were stained for Bcrp and Mrp2 proteins. Bar, 50 μm.

FIG. 8. Immunofluorescent colocalization analysis of Mrp2/Bcrp and Mrp2/Mrp4 proteins in fetal membranes. Double indirect immunofluorescence against Bcrp (green, A) and Mrp2 (red, B) proteins was performed on day 14 fetal membrane cryosections. Images A and B were merged to create image C, demonstrating colocalization of Bcrp and Mrp2 at the apical surface. Double indirect immunofluorescence against Mrp4 (green, D) and Mrp2 (red, E) proteins was similarly performed on day 14 fetal membrane cryosections. Images D and E were merged to create image F, demonstrating distinct localization of Mrp4 and Mrp2 to the basolateral and apical surfaces, respectively. Magnification, 40×; bar, 50 μm.

FIG. 9. Summary of xenobiotic transporter expression in mouse placenta and fetal membranes. The mouse placenta expresses a number of uptake (Oatp1a5, 2a1, 3a1, 4a1, and 5a1) and efflux (Mrp1, Mrp5, Mdr/Pgp, and Bcrp) transporters. Kalabis et al. (2007) demonstrated localization of Bcrp to the apical surface of the syncytiotrophoblasts in mouse placenta. Localization of Oatp, Mrp, and Mdr transporters in mouse placenta is currently unknown. The amniotic membrane and inverted yolk sac enclose the fetus and are together known as the fetal membranes. Endoderm-derived epithelial cells of the yolk sac express transporters on the apical/maternal face (Mrp2 and Bcrp) and the basolateral/fetal face (Mrp4, 5, and 6) (see inset). Mrp4, Mrp5, Mate-1, and Oatp1a5 transporters are also detected in the fetal membranes, although their cellular localization is unknown.


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