EXPRESSON AND FUNCTION OF ABCB1 AND ABCG2 IN HUMAN PLACENTAL TISSUE

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ABSTRACT:

The placenta plays an important role in modulating xenobiotic passage from mother to fetus. Studies in mice have demonstrated that placental ABCB1 and ABCG2 can affect the transfer of drugs across the placental barrier, suggesting a role for these transporters in protecting the fetus from environmental toxicants or drugs ingested by the mother during pregnancy. To assess the role of these transporters in the human placenta, studies were conducted to evaluate the expression and functional activity of placental ABCB1 and ABCG2. The effect of maternal smoking on these placental transporters was also assessed. Uptake rates of [3H]vinblastine and [3H]mitoxantrone were used to measure ABCB1 and ABCG2 activity, respectively, and CYP1A1 activity was assessed using ethoxyresorufin O-deethylation as a positive control for smoking-related enzyme induction. ABCB1 and ABCG2 expression levels were measured by immunoblotting techniques. ATP-dependent uptake of [3H]vinblastine in vesicles was osmotically sensitive, suggesting intravesicular accumulation, and was inhibited by verapamil, an ABCB1 inhibitor. ATP-dependent uptake of [3H]mitoxantrone was inhibited by fumitremorgin C, an ABCG2 inhibitor, but not by verapamil, suggesting that the uptake of [3H]mitoxantrone was primarily mediated by ABCG2. Although CYP1A1 activity was greatly induced in smokers, no statistical differences (p > 0.05) were noted in ABCB1 and ABCG2 activity or expression between smokers and nonsmokers. In summary, both ABCB1 and ABCG2 are expressed at high levels in human placenta and are functionally active, suggesting a protective role with respect to fetal exposure to xenobiotics ingested by the mother.

P-glycoprotein/ABCB1, encoded by the ABCB1 (MDR1; multidrug resistance) gene, is an efflux transporter that pumps xenobiotics out of cells, utilizing ATP as the energy source. Due to this mode of action, ABCB1 confers multidrug resistance capability to tumor cells against a wide spectrum of anticancer agents. In addition to cancer tissues, ABCB1 is also present in various normal tissues (Cordon-Cardo et al., 1990) such as intestine, liver, and kidney, where it plays an important role in absorption, distribution, and excretion of drugs (Bellamy, 1996). In addition, ABCB1 plays a role in the transport of xenobiotics across the blood-brain barrier, modulating their central nervous system effects (Schinkel et al., 1996). Another tissue in which ABCB1 regulates xenobiotic transfer is the placenta. The placenta provides a link between mother and fetus, playing a critical role in the provision of nutrition and growth factors that are necessary for development of the fetus. Drug efflux transporters such as ABCB1, multidrug-resistant associated proteins, and breast cancer resistance protein (BCRP) are known to be expressed in the placenta and may play a protective role for the developing fetus.

Studies in mdr knockout mice have demonstrated that placental ABCB1 can affect the transfer of xenobiotics across the placental barrier (Lankas et al., 1998; Smit et al., 1999), suggestive of a role in protecting the fetus from drugs ingested by the mother during pregnancy or from environmental toxicants. Lankas et al. (1998) have demonstrated that when pregnant dams were exposed to ivermectin, the fetuses of wild-type mice with abundant ABCB1 were totally insensitive to this teratogen, whereas the fetuses of mdr knockout mice that were deficient in ABCB1 developed cleft palate. Similarly, Smit et al. (1999) demonstrated that mice in which both the mdr genes, mdr1a and mdr1b, were disrupted had a 2.4-, 7-, and 16-fold higher transplacental transport of ABCB1 substrates such as digoxin, saquinavir, and paclitaxel, respectively, as compared with wild-type mice. These investigators further demonstrated that after oral administration of ABCB1 inhibitors like PSC833 (valspodar) or GG918 (N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isouinolinyl)-ethyl]-phenyl]-9,10-dihydro-5-methoxy-9-oxo-4-acyclic carboxamine), drug levels in wild-type mice were comparable to those in knockout mice.

ABC2G, also known as ABCP (Allikmets et al., 1998)/MXR (Miyake et al., 1999)/BCRP (Doyle et al., 1998), is a 70-kDa protein encoded by ABCG2 and is also involved in the active efflux of drugs. It is expressed at high levels in the placenta, liver, and small intestine, with lesser expression observed in the kidney, heart, and brain (Doyle

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ABBREVIATIONS: P-gp, P-glycoprotein; CNS, central nervous system; mdr, multidrug resistance; PAH, polycyclic aromatic hydrocarbon; CYP1A1, cytochrome P450 1A1; 3-MC, 3-methylcholanthrene; ABC superfamily, ATP-binding cassette superfamily; ABCP, ABC transporter in placenta; BCRP, breast cancer resistance protein; MXR, mitoxantrone resistance gene; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.

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Although animal data suggest that placental ABCB1 and ABCG2 may serve a protective role for the developing fetus, their role in human placenta is not well defined. A major source of concern with respect to the fetus is the exposure to environmental toxican. Polycyclic aromatic hydrocarbons (PAHs) are primary constituents of cigarette smoke, and cigarette smoking has been associated with adverse outcomes during pregnancy, including changes in function and structure of the placenta (Sastry and Janson, 1995). Smoking and the associated PAHs can affect drug-metabolizing enzymes and increase levels of CYP1A1 in human placenta (Pasanen and Pelkonen, 1990). Studies have suggested that PAHs may also affect the expression and activity of efflux transporters, such as ABCB1 (Schuetz et al., 1998; Maliepaard et al., 2001). Studies in

The present study was initiated to characterize the expression and functional activity of both ABCB1 and ABCG2 in human placenta. In addition, the effect of maternal smoking on expression and functional activity of these two transporters was evaluated. The results of these studies are described herein.

Materials and Methods

Materials. [3H]Vinblastine (10.9 Ci/mmole) and [3H]mitoxantrone (3 Ci/mmole) were purchased from Amersham Biosciences Inc. (Piscataway, NJ) and American Radiolabeled Chemicals (St. Louis, MO), respectively. Verapamil, mitoxantrone, vinblastine, resorufin, 7-ethoxyresorufin, ATP, creatine phosphokinase, NADPH, and bovine serum albumin were purchased from Sigma-Aldrich (St. Louis, MO). Creatine phosphate was purchased from Fluka (Buchs, Switzerland). Scintiverse BD scintillation cocktail and magnesium chloride were purchased from Fisher Scientific Co. (Pittsburgh, PA). Fumitremorgin C (FTC) was a gift from Dr. Susan Bates (National Institutes of Health, Bethesda, MD). All other chemicals were purchased from commercial sources and were of the highest purity available.

Tissue Collection. Human placental tissue was obtained under a protocol approved by the West Virginia University Institutional Review Board for the Protection of Human Research Subjects. Placentas were obtained from 10 smokers and 10 nonsmokers who had given written informed consent prior to obstetric delivery. Smoking status was assessed by patient interview. Placentas were collected and processed immediately after delivery. Two triangular wedges extending from the center of the placenta to the placental margin were cut. One piece was frozen at −80°C for later preparation of microsomes, and the other piece was immediately processed to prepare the membrane vesicles.

Preparation of Microvillus Membrane Vesicles. Microvillus membrane vesicles were prepared according to the methods of Illsley et al. (1990). The final membrane pellet was resuspended in buffer containing 10 mM Tris-HCl (pH 7.4) and 250 mM sucrose with a 25-gauge needle through which the suspension was passed 10 times. The vesicles were aliquoted and stored at −80°C. Protein concentration was determined by the BCA assay kit (Pierce Chemical, Rockford, IL). The percentage of inside-out vesicles was determined by sialidase accessibility (Warren, 1959; Aminoff, 1961), and membrane vesicles were found to be 18 to 20% inside-out.

Uptake by Membrane Vesicles. ATP-dependent transport of the radiolabeled substrate into membrane vesicles was measured by filtration with a 12-channel sampling manifold (Millipore Corporation, Billerica, MA). Transport buffer (45 µL) containing 10 mM Tris-HCl, pH 7.4, 250 mM sucrose, 10 mM MgCl2, 20 nM [3H]vinblastine, or 50 nM [3H]mitoxantrone, enough of each unlabeled drug to produce a final concentration of 100 nM, and an ATP-regenerating system (10 mM phosphocreatine and 100 µg/ml creatine kinase) were warmed to 37°C before initiating the reaction. Reactions were carried out in the presence or absence of 4 mM ATP. The reaction was initiated by addition of 10 µL of vesicle aliquot (25 µg of protein) and quenched after 10 min (vinblastine) or 6 min (mitoxantrone) by the addition of 3 mL of ice-cold stop solution (10 mM Tris-HCl, pH 7.4, and 250 mM sucrose). The quenched reaction mixture was filtered immediately through nitrocellulose filters (0.45-µm; Whatman, Clifton, NJ) presoaked in 3% bovine serum albumin and washed, under light suction, with an additional 3 mL of ice-cold stop solution. The filters were then placed in a scintillation vial, Scintiverse BD (Fisher Scientific Co.) scintillant was added, and radioactivity was measured by liquid scintillation counting. ATP-dependent uptake of radiolabeled substrate was calculated as the difference in the uptake of substrate measured in the presence and absence of ATP, and is reported as picomoles per milligram of protein.

Immunoblotting for ABCB1 and ABCG2. Membrane vesicle protein (10 µg) was heated at 90°C for 10 min, and then loaded onto 7% Tris-acetate mini-gels (Invitrogen, Carlsbad, CA) and electrophoresed. The proteins were then transferred to a nitrocellulose membrane, after which the membrane was blocked with blocking buffer (5% nonfat dry milk in Tris-buffered saline) for 1 h at room temperature and then incubated with C-219 ABCB1 antibody or BXP-21 ABCG2 antibody (1:1000; Signet Laboratories, Dedham, MA) overnight at 4°C. The membrane was then washed with Tris-buffered saline containing 0.1% Tween 20 and incubated with horseradish peroxidase-linked sheep anti-mouse antibody (1:5000; Kirkegaard and Perry Laboratories, Gaithersburg, MD) for 1 h at room temperature. After washing with Tris-buffered saline containing 0.1% Tween 20, the blots were developed with an enhanced chemiluminescence detection system (Amersham Biosciences Inc.). Although equal amounts of protein were loaded per lane, the data were normalized with actin as a loading control to account for any potential differences in protein loading. The membranes, which were probed for ABCB1 and ABCG2, were washed with wash buffer and stripped using stripping buffer. The stripped membranes were then probed with anti-actin antibody (1:200; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), followed with horseradish peroxidase-linked human anti- goat antibody (1:2000; Santa Cruz Biotechnology, Inc.). The relative amounts of ABCB1, ABCG2, and actin were determined by density measurements made with Optimas Imaging software (Media Cybernetics, Inc., Silver Spring, MD), and the relative amounts of ABCB1 and ABCG2 were normalized with actin and reported as optical density values.

Measurement of CYP1A1 Activity. Placental microsomes were prepared according to established methods (Vaz et al., 1992). Ethoxyresorufin-O-deethylation (EROD) activity catalyzed by CYP1A1 was determined as described by Burke et al. (1985), with slight modifications. Briefly, 0.5 mg/ml microsomal protein was incubated with 20 µM 7-ethoxyresorufin (2 mM stock in dimethyl sulfoxide) and Na-potassium phosphate buffer (0.1 M, pH 7.6) in a total volume of 1.25 ml at 37°C for 1 min. One milliliter of the reaction mixture was transferred to a cuvette, and the reaction was initiated by addition of 1 mM NADPH. Relative fluorescence intensity was monitored for 4 min using a Shimadzu RF-5301PC spectrophotofluorometer Shimadzu (Shimadzu, Kyoto, Japan) at 37°C, set at excitation and emission wavelengths of 530 nm and 585 nm, respectively. The rate of formation of resorufin from 7-ethoxyresorufin was calculated from a standard curve for resorufin, and activity is reported as pmol/min/mg protein.

Statistical Analysis. Statistical comparisons of ABCB1, ABCG2, and CYP1A1 activity, as well as ABCB1 and ABCG2 expression between smokers and nonsmokers, were made using a one-sided t test. Statistical significance was set at p < 0.05.

Results

ABCB1 and ABCG2 Expression in Human Placenta. A 170-kDa ABCB1 protein was detected in all 20 samples of human placenta. However, substantial interindividual variability in ABCB1 expression among the samples was noted (Fig. 1). Statistical analysis showed that ABCB1 protein abundance was comparable between smokers and nonsmokers (Table 1). Similarly, an ABCG2 protein band was detected at 70 kDa, and its expression also was comparable between smokers and nonsmokers (Table 1).

Uptake of [3H]Vinblastine in Membrane Vesicles (ABCB1 activity). ATP-dependent transport of [3H]vinblastine was observed in membrane vesicles isolated from human placenta. This transport was also time-dependent (data not shown). To determine whether the
transport was truly intravesicular or simply due to increased binding to ABCB1 in the presence of ATP, the effect of osmolarity on the transport of vinblastine by ABCB1 was studied in the presence and absence of ATP. Figure 2 demonstrates that the ATP-dependent uptake observed was intravesicular, since the uptake decreased with decreasing intravesicular volume resulting from increased osmolarity.

To determine whether vinblastine uptake was mediated by ABCB1, uptake studies were carried out in a representative placental sample, in the presence of verapamil, a known inhibitor of ABCB1. ATP-dependent vinblastine uptake was inhibited 42% by 10 μM and 71% by 100 μM verapamil (Fig. 3), confirming the involvement of ABCB1 in vinblastine uptake.

Placental ABCB1 activity, measured as the uptake of vinblastine, was not statistically different (p = 0.175) in smokers and nonsmokers (Table 2). With respect to the correlation of ABCB1 expression and activity, a positive correlation, r = 0.8 and 0.75, was obtained in smokers and nonsmokers, respectively.

Uptake of [3H]Mitoxantrone in Membrane Vesicles (ABCG2 Activity). ATP-dependent transport of mitoxantrone was observed in membrane vesicles. To determine whether this transport was mediated by ABCG2, mitoxantrone uptake was measured in the presence of fumitremorgin C, a selective inhibitor of ABCG2, as well as the ABCB1 inhibitor verapamil, in a representative placental sample. In the presence of 10 μM fumitremorgin C, a 65% inhibition of ATP-dependent mitoxantrone transport was observed, whereas 10 μM verapamil had minimal effect (Fig. 4), suggesting that [3H]mitoxantrone transport was primarily an ABCG2-mediated process. Placental ABCG2 activity, measured as the uptake of mitoxantrone, was not statistically different (p = 0.388) between smokers and nonsmokers (Table 2).

ABCG2 is a half-transporter and is thought to require dimerization to be active (Ewart and Howells, 1998). It has been demonstrated that in the absence of reducing agent, ABCG2 migrates as a 140-kDa protein on electrophoretic gels (Kage et al., 2002). We observed similar results on blots where ABCG2 migrated as a 140-kDa protein under nonreducing conditions [in the absence of dithiothreitol (DTT)] and as a 70-kDa protein under reducing conditions (in the presence of DTT) (Fig. 5). This suggests that ABCG2 exists as a dimer in nonreducing conditions and as a monomer under reducing conditions. In addition, we were able to demonstrate for the first time that there was decreased ATP-dependent transport of mitoxantrone by ABCG2 in the presence of 1 mM DTT, as compared with the absence of DTT (Fig. 6). Thus, in the presence of DTT, ABCG2 appears to be present as a monomer and loses its ability to transport substrates, confirming that the intact dimer is required for functionality.

EROD in Placental Microsomes (CYP1A1 Activity). As a positive control for the effects of smoking, we measured CYP1A1 function using the EROD assay. CYP1A1 activity was highly induced in smokers; however, we were only able to measure this activity in 2 of 10 micro-

![Fig. 1. Representative immunoblot of ABCB1 in placental vesicles. Lanes 1, 3, 9, 11, and 12 are samples from smokers, and lanes 2, 4, 5, 6, 7, 8, and 10 are samples from nonsmokers.

![Fig. 2. Effect of osmolarity on ATP-dependent uptake of [3H]vinblastine in microvillus membrane vesicles. Placental vesicles (25 μg) were incubated for 10 min with [3H]vinblastine in the presence or absence of ATP (4 mM) in transport buffer containing increasing concentrations of sucrose (250, 333, 500, and 1000 mM). Data points represent the absolute difference between the mean values of triplicate measurements of [3H]vinblastine uptake made in the absence and presence of ATP. The coefficient of variation within the triplicates ([3H]vinblastine uptake either in the presence of ATP or the absence of ATP) was less than 15%.

<p>| TABLE 1 | Optical density values (mean ± S.D.) for ABCB1 and ABCG2 protein abundance normalized against actin in human placental tissue from smokers and nonsmokers |</p>
<table>
<thead>
<tr>
<th>Densitometric Analysis</th>
<th>Optical Density</th>
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</thead>
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<tr>
<td></td>
<td>Smokers (n = 10)</td>
</tr>
<tr>
<td>ABCB1 expression</td>
<td>4.26 ± 1.30</td>
</tr>
<tr>
<td>ABCG2 expression</td>
<td>3.79 ± 1.71</td>
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</table>
somal samples prepared from placentas of nonsmokers. Table 2 shows the activity seen in each group and the range of values obtained.

**Discussion**

The role of ABCB1 as an efflux transporter in tumor cells has been extensively studied. Expression of ABCB1 also has been demonstrated in normal human tissues such as intestine, liver, and kidney, wherein it plays a role in absorption, distribution, and elimination of drugs. Studies in mice have demonstrated the protective function that ABCB1 plays at the blood-brain barrier and in the placenta. More recently, the transporter ABCG2 has increasingly been recognized as playing a prominent role in both tumor and normal tissues. These transporters are expressed at the apical surface of placental trophoblast, and given their efflux nature, they can transport xenobiotics back to the mother, thus serving a protective role for the developing fetus. During pregnancy, the placenta may be exposed to various xenobiotics including drugs ingested by the mother or environmental toxicants. It has been suggested that PAHs, which are toxicants contained in cigarette smoke, may affect efflux transporters, but this has not been studied in the human placenta. To evaluate the expression and activity of both ABCB1 and ABCG2, placental tissue was collected from both women who abstained from smoking during their pregnancy and those who continued to smoke throughout pregnancy, and transporter function and expression were evaluated. The results of these studies demonstrated that both ABCB1 and ABCG2 are extensively expressed in human placenta and are functionally active, but are unaffected by cigarette smoking.

Vinblastine was used as a model substrate of ABCB1 transport in vesicles derived from human placental tissue. Studies with increasing medium osmolarity confirmed that the radioactivity measured was 527

![FIG. 3. Effect of verapamil (VER) on ATP-dependent uptake of vinblastine in microvillus membrane vesicles from a representative placental sample. Data represent the absolute difference between the mean values of triplicate measurements of [3H]vinblastine uptake made in the absence and presence of ATP, both with and without verapamil (10 µM or 100 µM). The coefficient of variation within the triplicates ([3H]vinblastine uptake either in the presence of ATP or the absence of ATP) was less than 15%.](image)

![TABLE 2](image)

<table>
<thead>
<tr>
<th>Parameter (Units)</th>
<th>Smokers (n = 10)</th>
<th>Nonsmokers (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCB1-mediated vesicular uptake of vinblastine (pmol/mg protein)</td>
<td>2.25 ± 1.12</td>
<td>1.72 ± 0.67</td>
</tr>
<tr>
<td>ABCG2-mediated vesicular uptake of mitoxantrone (pmol/mg protein)</td>
<td>6.25 ± 1.55</td>
<td>5.53 ± 2.09</td>
</tr>
<tr>
<td>CYP1A1 activity in placental microsomes (pmol/min/mg protein)</td>
<td>3.61 ± 2.96</td>
<td>0.56</td>
</tr>
</tbody>
</table>

a Measurable values; 8 of the 10 samples were below the limit of detection.

[FIG. 4. Effect of an ABCG2 inhibitor (FTC) and an ABCB1 inhibitor (verapamil (VER)) on the ATP-dependent uptake of mitoxantrone in microvillus membrane vesicles from a representative placental sample. Data represent the absolute difference between the mean values of triplicate measurements of [3H]mitoxantrone uptake made in the absence and presence of ATP, both with and without FTC (10 µM or 100 µM). The coefficient of variation within the triplicates ([3H]mitoxantrone uptake either in the presence of ATP or the absence of ATP) was less than 15%. FTC inhibited mitoxantrone uptake by 65%, whereas verapamil had minimal effect on mitoxantrone transport, suggesting that mitoxantrone transport is predominately mediated by ABCG2.](image)
due to intravesicular transport and not nonspecific binding to the vesicles. Furthermore, the inhibition of this process by the prototypical ABCB1 inhibitor, verapamil, confirmed that ABCB1 active transport was being measured. Substantial expression and activity were noted in vesicles prepared from placental tissue, confirming its importance in human placental regulation of xenobiotic transport. Hitzl et al. (2004) recently studied the expression of ABCB1 in human placenta and its association with polymorphisms in the MDR1 gene. These authors reported 8-fold variability in ABCB1 protein expression, similar to the 7-fold variability noted in the current study. It is of note that no differences were noted in either expression or activity of ABCB1 in tissue from smokers as compared with tissue from non-smokers (Tables 1 and 2). The observation of increased CYP1A1 activity served as a positive control for induction of enzymes by smoking and confirmed that the similar expression and activity of ABCB1 between smokers and nonsmokers was due to lack of effect of smoking on this process and not due to inadequate smoking exposure. These results were somewhat surprising in light of the observation that PAHs, such as 3-methylcholanthrene and TCDD, can induce ABCB1 expression in human hepatocytes (Schuetz et al., 1995). Although cigarette smoke is known to contain a variety of PAHs, it is possible that this effect is only mediated by 3-methylcholanthrene or other PAHs not contained in cigarette smoke; that the PAH dose was too low to induce ABCB1, although it was satisfactory.
for CYP1A1 induction; or that the regulatory elements necessary for ABCB1 induction are not present in the placenta but are in hepatocytes. Regardless of the reason for lack of PAH induction of ABCB1, it appears that maternal smoking would not be expected to affect the placental transport of xenobiotics by ABCB1.

In addition to ABCB1, ABCG2 also has been reported to be present at the apical surface of placenta. In vitro studies in polarized mammalian cell lines, in which the murine homolog of ABCG2 was expressed, demonstrated apical transport of topotecan and mitoxantrone (Jonker et al., 2000). Studies in mdr knockout mice have suggested that, similar to ABCB1, ABCG2 is also likely to reduce the fetal exposure to some drugs. Jonker et al. (2000) demonstrated that uptake of topotecan was 3.2-fold higher in the fetuses of mdr knockout mice in the presence of GF120918 (N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isouinolinyl)ethyl]-phenyl]-9,10-dihydro-5-methoxy-9-oxo-4-acridine carbamamide), an ABCG2 inhibitor, as compared with fetuses of vehicle-treated mdr knockout mice. To the best of our knowledge, the present studies constitute the first report of functional studies of ABCG2 in human placenta. Kobayashi et al. (2005) recently published data on ABCG2 expression and polymorphisms in 100 placenatlas, but did not measure functional activity. These authors reported a 4- to 5-fold variability in ABCG2 expression in different haplotypes, similar to the 5-fold variability in ABCG2 expression noted in the 20 human placental samples used in the current study. Mitoxantrone, a known substrate for ABCG2, was used to measure ABCG2 function in the murine villus membrane vesicles. The sole involvement of ABCG2 in mitoxantrone transport was verified by observing inhibition of mitoxantrone uptake in the presence of fumitremorgin C, a selective inhibitor of ABCG2 (Rabindran et al., 1998), and a lack of inhibition by the ABCB1 inhibitor, verapamil. Again, substantial expression and activity of ABCG2 were observed in vesicles derived from human placental tissue. In fact, transport by ABCG2 appeared to be higher than ABCB1 transport in human placental tissue. However, as observed with ABCB1, smoking appeared to have no effect on either the expression or activity of ABCG2, suggesting that mothers who smoke do not have altered ABCG2 transport as compared with those who do not smoke.

ABCG2 is a half-transporter with 6 transmembrane domains and only one ATP-binding site, unlike ABCB1 which is a full transporter with 12 transmembrane domains and two ATP-binding sites (Kage et al., 2002). Since ABCG2 is a half-transporter, it is assumed to require dimerization to be a functional protein (Ewart and Howells, 1998). In its monomeric form, ABCG2 migrates as a 70-kDa protein. However, under nonreducing conditions, ABCG2 migrates as a 140-kDa protein, suggesting it forms a dimer (Kage et al., 2002). To confirm this dimerization, placental vesicle samples were electrophoresed under reducing (in the presence of DTT) and nonreducing conditions (in the absence of DTT) (Fig. 5). Under nonreducing conditions, a large band was observed at 140 kDa. However, under reducing conditions, this 140-kDa band was absent, and only a single 70-kDa protein band was observed, suggestive of the monomer. It should be noted that minimal expression of the 70-kDa protein was observed under nonreducing conditions, possibly due to some ABCG2 monomers that had not dimerized. To confirm that dimerization of ABCG2 is necessary for functionality, we performed mitoxantrone uptake studies in the presence and absence of 1 mM DTT (Fig. 6). Since DTT is a reducing agent, it should cleave the dimer and thus decrease the functional activity of ABCG2. An approximately 65% reduction in mitoxantrone uptake was noted in the presence of DTT as compared with the absence of DTT. These results suggest that ABCG2 dimerization is required for functionality. Since no dimer was detected in the immunoblots obtained in the presence of DTT (reducing conditions), the small amount of mitoxantrone transport noted in the presence of 1 mM DTT was most likely due to the binding of mitoxantrone to ABCG2 in the presence of ATP.

In summary, we were able to readily measure ABCB1 and ABCG2 expression and activity in placental membrane vesicles, demonstrating their role in placental xenobiotic transport. Additionally, smoking appeared to have no effect on expression or activity of either transporter, suggesting that women who smoke during pregnancy are not at risk for altered xenobiotic transport, at least by these two transporters, as compared with nonsmoking pregnant women. Future studies will be directed at quantitating the role these transporters play in xenobiotic disposition and fetal exposure in the intact feto-placental unit.

References

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