International Immunopharmacology 26 (2015) 349–356

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Maternal bacterial infections impact expression of drug transporters in human placenta

Vanja Petrovic, Dea Kojovic, Alex Cressman, Micheline Piquette-Miller *

Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada

ARTICLE INFO

Article history:
Received 1 December 2014
Received in revised form 17 March 2015
Accepted 10 April 2015
Available online 21 April 2015

Keywords:
Drug transporters
Infection
Chorioamnionitis
Placenta
Inflammation
Cytokines

ABSTRACT

Background: Several efflux and uptake transporters in the placenta are involved in the transmembrane transport of endogenous substrates and xenobiotics. Their expression and function may be altered in maternal complications associated with inflammation. Our objective was to examine the effect of chorioamnionitis, a bacterial intra-amniotic infection on the expression of clinically important transporters in human placenta.
Methods: Human placental samples were collected from preterm and term pregnancies diagnosed with chorioamnionitis infection and were gestational age-matched with samples from pregnancies with no obstetric complications, using predefined exclusion criteria. Transporter protein expression was quantified using Western blots while cytokine and transporter mRNA expression was measured via real-time polymerase chain reaction.
Results: mRNA levels of pro-inflammatory cytokines IL-6, IL-1β and TNF-α were markedly elevated by 2.5- to 3-fold in preterm placentas with infection, relative to preterm controls ($p < 0.05$). Expression of ABCG2 and SLCO2B1 was downregulated by 48 to 57% ($p < 0.05$) in placentas from women with infection and preterm parturition, relative to preterm healthy controls. Protein and mRNA expression changes were generally consistent. At term, *ABCG2* mRNA and SLCO2B1 protein expression levels were significantly downregulated, relative to controls. Significant changes in ABCB1 and SLCO4A1 expression were not observed, however *ABCB1* transcript levels strongly correlated with IL-6, IL-1β and TNF-α expression ($p < 0.001$), potentially suggesting involvement of cytokine-mediated regulation.
Conclusions: Collectively, these data show that maternal infections impact the expression of key drug transporters in placenta, suggesting that materno-fetal drug transport may be altered by changes in placental expression of ABC and OATP transporters.

1. Introduction

During intrauterine life, the placenta is involved in nutrient uptake and serves as a barrier against potentially harmful substances. Several ATP-binding-cassette (ABC) efflux transporters as well as solute carrier (SLC) uptake transporters are highly expressed in the syncytiotrophoblast layer of the placenta and are believed to control fetal exposure to numerous endogenous and exogenous substances. These transporters are involved in the transport of a wide variety of chemically diverse substrates, ranging from bulky lipophilic drugs and toxins to organic anions, carbohydrates, and bile acids [1]. Previous studies in animal models and cell lines demonstrated that pro-inflammatory cytokines and inflammatory conditions can alter the expression of ABC and OATP transporters (reviewed in [2–4]). However, the potential impact of maternal inflammation on placental drug transport and fetal exposure has only recently gained attention.

The ABC efflux transporters, multidrug resistance protein 1 (MDR1; ABCB1; P-glycoprotein) and breast cancer resistance protein (BCRP; ABCG2) are of particular importance in regard to fetal drug exposure since they are abundantly expressed in the placenta and have been implicated in limiting the placental transport of many clinically important drugs (reviewed in [5,6]). Relative to other tissues, the basal expression of ABCG2 is highest in the placenta [7]; it is also the most highly expressed ABC transporter in this tissue, implying its functional importance [8]. Both ABCG2 and ABCB1 are localized in the apical surface of syncytiotrophoblasts functioning as a protective mechanism for the fetus by actively removing substrates passing from maternal circulation and clearing endogenous substrates entering from the fetal circulation [9,10].

Abbreviations: ABC transporter, ATP-binding cassette transporter; BCRP, breast cancer resistance protein (ABCG2); CTL-PT, control-preterm; CTL-T, control-term; IL-1β, interleukin-1β; IL-6, interleukin-6; INF-PT, infection-preterm; INF-T, infection-term; MDR1, multidrug resistance protein 1 (ABCB1P-glycoprotein); OATP, organic anion transporting polypeptide (SLCO); qRT-PCR, quantitative reverse-transcriptase polymerase chain reaction; SLC, solute carrier; TBST, Tris-buffered saline/Tween 20; TNF-α, tumor necrosis factor-α.

* Corresponding author at: Leslie Dan Faculty of Pharmacy, University of Toronto, 144 College Street, Toronto, Ontario, M5S 3M2, Canada. Tel.: +1 416 946 3057; fax: +1 416 978 8511.
E-mail address: m.piquette.miller@utoronto.ca (M. Piquette-Miller).

http://dx.doi.org/10.1016/j.intimp.2015.04.020
1567-5769/

The placenta is also involved in the transport of steroid and thyroid hormones, which are necessary for maintaining pregnancy and for normal fetal development. In the placenta, the organic anion transporting polypeptide (OATP) 2B1 (SLCO2B1) mediates the basolateral uptake of sulfated steroid conjugates [11] such as the estrogen precursor, dehydroepiandrosterone sulfate (DHEAS) and estrone 3 sulfate metabolite. Apically expressed OATP4A1 (SLCO4A1) mediates the placental uptake of thyroid hormones essential for normal fetal growth and neurodevelopment [12]. SLCO4A1 and SLCO2B1 and are two of the most highly expressed OATPs found in human placenta, yet very little information exists regarding their placental regulation [7]. These two OATP family members are also involved in the transport of several clinically relevant drugs [1].

Inflammatory effects on the expression of transporters in placenta have been investigated in animal models. Studies in endotoxin-treated pregnant rodents reported inflammation-mediated decreases in the placental expression of several ABC and OATP transporters, with corresponding changes in their activity and evidence of altered fetal drug exposure [13–15]. However, data on the effect of inflammation and infection on drug transport in pregnant women is limited, even though many prevalent obstetric complications are associated with an inflammatory response. For example, infections are known to result in highly elevated levels of pro-inflammatory cytokines such as IL-6 in maternal serum and amniotic fluid [16]. Maternal bacterial vaginosis is reported to occur in as high as 6–32% of pregnancies, Chlamydia occurs in 2–21% of pregnancies and urinary tract infections resulting from streptococcal bacteriuria afflict 2–4% of pregnancies [17,18]. Such bacterial infections may result in chorioamnionitis, a serious obstetric complication involving inflammation of the placenta and the placental amnion and chorion, which complicates up to 4% of all births, including 1–2% of term and up to 15% of preterm births [19,20]. Recently, increased transcript levels of *ABCB1* and *ABCG2* were reported in placentas from preterm labor with inflammation, a finding which was correlated with increased IL-8 levels [21]. It is still unknown whether there are inflammation-mediated changes in the placental expression of these transporters at term and whether expression of the OATP transporters is also affected. As protein expression is often more highly correlated with functional changes in transport, our objective was to further investigate the mRNA and protein expression of ABCG2 and ABCB1, as well as SLCO2B1 and SLCO4A1 transporters in placentas obtained from preterm and term pregnancies complicated by maternal infection and inflammation. Chorioamnionitis was chosen as a suitable example of a maternal infection, due to its prevalence and significance in obstetrics. These transporters were examined based on their importance in the transplacental transfer of clinically important endogenous and exogenous substrates. Furthermore, the transport of certain substrates is thought to be co-regulated by pairs of uptake and efflux transporters. For example, substrate specificity is shared between BCRP and OATP2B1 and their coordinated activity is believed to play an important role in transplacental transfer [22]. The syncytiotrophoblast localization of the transporters we examined is illustrated in Fig. 1.

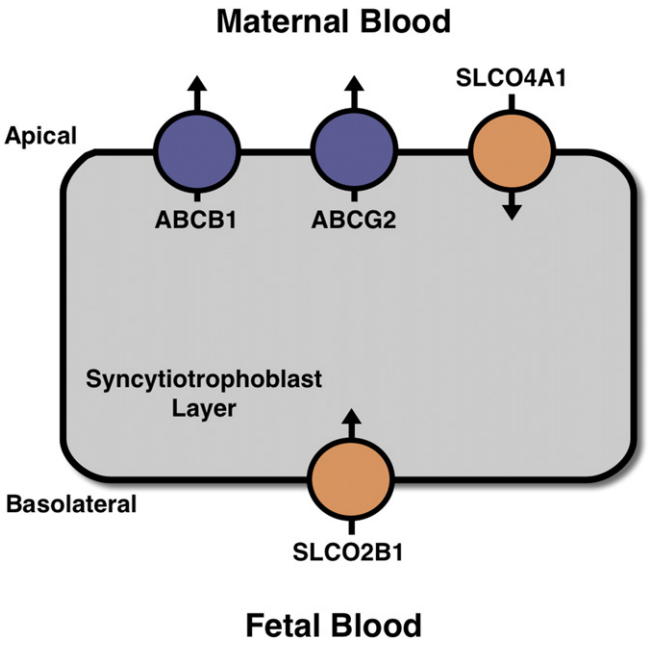

Fig. 1. Illustration of localization of ABCB1, ABCG2, SLCO2B1 and SLCO4A1 in the placenta. The syncytiotrophoblast cell layer of the placenta expresses a number of apical and basolateral transport proteins that contribute to the function of the placental barrier, including the SLC uptake transporters SLCO2B1 and SLCO4A1 and the ABC efflux transporters ABCB1 and ABCG2 [8].

2. Materials and methods

2.1. Sample acquisition

Fifty human placental specimens from pregnancies meeting our inclusion/exclusion criteria were obtained by the Research Centre for Women's and Infants' Health (RCWIH) BioBank program at Mount Sinai Hospital, Toronto, Canada, in accordance with the policies of the Mount Sinai Hospital Research Ethics Board and following the tenets of the Declaration of Helsinki. Sample acquisition, placental processing and measurement are detailed on the RCWIH BioBank's website: http://biobank.lunenfeld.ca, as previously described [23]. Placental samples were grouped by chorioamnionitis infection from term and preterm pregnancies (INF-T and INF-PT) or control term and preterm pregnancies (CTL-T and CTL-PT). Pathologic changes accompanying cases of chorioamnionitis were examined and classified according to the criteria set out by Redline and colleagues [24]. This classification system includes 3 stages of disease progression (3 being the highest) with 2 grades of intensity at each stage, for both the maternal and the fetal inflammatory responses. Control placental samples were collected from healthy pregnancies matched for gestational age (term or preterm). All clinical data that were extracted from patient charts and collected by the RCWIH BioBank program of Mount Sinai Hospital was made available to this study. Extensive review of clinical data was completed to ensure that all patients met our inclusion/exclusion criteria and that any medications that the patients were taking were not known inhibitors or inducers of the studied transporters. Women were excluded from the control group if they had any concurrent conditions that could impact the expression of transporters, such as various endocrine, cardiovascular, hematological, neurological, inflammatory, autoimmune, renal or hepatic complications, as well as women with diagnosed infections, obstetric complications (including preeclampsia, gestational diabetes and other complications), cancer survivors, or those that reported smoking or recreational drug use during pregnancy (detailed in supplementary data, Table S1). Similar exclusion criteria were applied for the INF group, apart from the diagnosed intra-amniotic infection that constitutes chorioamnionitis. Samples included in this study reflect RCWIH BioBank availability between 2010 and 2013.

2.2. Western blotting and transporter protein expression

Crude membrane fractions were isolated from 300 mg of placental tissue. Samples were homogenized using a motorized pestle in lysis buffer (0.1 M Tris–HCl, pH 7.5, containing Protease Inhibitor Cocktail 1–3 μl/ml and Phenylmethylsulfonyl fluoride 50 μg/ml; Sigma-Aldrich, Oakville, ON). Homogenates were centrifuged at 2000 *g* for 20 min and the resulting supernatant was re-spun at 100,000 *g* for 60 min at 4 °C. The resulting protein pellet was washed and dissolved in a small volume of lysis buffer. Total protein concentrations were quantified using the Bradford assay with BSA standards. Isolated protein samples (30–45 μg) in Laemmli sample loading buffer were heated at