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Impact of Bacterial and Viral Challenge on Multidrug Resistance in First- and Third-Trimester Human Placenta

Phetcharawan Lye,* Enrrico Bloise,*† Mohsen Javam,* William Gibb,‡§ Stephen J. Lye,*¶‖** and Stephen G. Matthews*¶‖**

From the Departments of Physiology, Obstetrics and Gynecology,¶ and Medicine,‖ University of Toronto, Toronto, Ontario, Canada; the Laboratory of Translational Endocrinology,† Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; the Departments of Cellular and Molecular Medicine‡ and Obstetrics and Gynecology,§ University of Ottawa, Ottawa, Ontario, Canada; and the Lunenfeld-Tanenbaum Research Institute,** Mount Sinai Hospital, Toronto, Ontario, Canada*

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Address correspondence to Stephen G. Matthews, Ph.D., Department of Physiology, Faculty of Medicine, University of Toronto, Medical Sciences Bldg., 1 King's College Circle, Toronto, ON M5S 1A8, Canada. E-mail: stephen.matthews@utoronto.ca.

The ABC transporters P-glycoprotein (P-gp, official gene symbol *ABCB1*) and breast cancer resistance protein (BCRP, official gene symbol *ABCG2*) protect the conceptus from exposure to toxins and xenobiotics present in the maternal circulation. Viral or bacterial challenges alter expression of placental multidrug transporters in rodents. We hypothesized that exposure to lipopolysaccharide (LPS, bacterial antigen) and polyinosinic—polycytidylic acid (poly(I:C), viral antigen) would decrease P-gp and BCRP in the human placenta. Placental explants from first and third trimesters were challenged with 0.1 to 10 μg/mL LPS or 1 to 50 μg/mL poly(I:C) for 4 or 24 hours; mRNA levels, protein expression, and localization were assessed by quantitative real-time PCR, Western blot analysis, and immunohistochemistry, respectively. Toll-like receptor (TLR)-3 and TLR-4 mRNA expression increased from the first to third trimester ($P < 0.01$), and the receptors localized to cytotrophoblasts in the first trimester and to syncytiotrophoblasts in the third trimester. LPS exposure in first-trimester explants decreased ($P < 0.001$) ABCB1 and ABCG2 mRNA and protein levels. In contrast, poly(I:C) decreased ($P < 0.05$) ABCB1, TLR-3, and TLR-4 mRNA levels in the third trimester but not first trimester. LPS and poly(I:C) treatments increased ($P < 0.01$) IL-8 and chemokine ligand 2. Results suggest that bacterial infections likely alter exposure of the conceptus to toxins and drugs during early pregnancy, whereas viral infections may disrupt fetal protection in later stages of pregnancy. *(Am J Pathol 2015, 185: 1666—1675; http://dx.doi.org/10.1016/j.ajpath.2015.02.013)*

The placenta supports the growth and development of the fetus through hormone production and by enabling the transport of oxygen and nutrients from mother to fetus. It also plays an important role in protecting the fetus from substances in the maternal blood that would otherwise be detrimental to the conceptus and its development, such as glucocorticoids and environmental toxins (eg, organophosphate pesticides and endocrine disruptors).[1—3] This barrier function is supported by a series of proteins within the trophoblast, including membrane-bound transporters (multidrug resistance proteins) that efflux unwanted factors that enter the trophoblast, back into the maternal circulation.[3]

Two multidrug transporter proteins, P-glycoprotein (P-gp; encoded by the *ABCB1* gene) and the breast cancer resistance protein (BCRP; encoded by the *ABCG2* gene), are enriched at the apical membrane of syncytiotrophoblast layer[3—6] and play an important role in protecting the fetus from exposure to steroids, environmental toxins, and xenobiotics.[3,7] We have reported that placental P-gp expression decreases progressively with advancing gestation, whereas placental BCRP levels increase toward term,[4—6] demonstrating a gestational age-dependent pattern of expression. Expression of these proteins is regulated by a number of factors, including placental hormones[8—10] and oxygen tension. In the case of oxygen, the effect on P-gp and BCRP expressions is gestational age dependent.[4,11]

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Table 1 List of Primers Used in This Study

Primer name	Sequence	Reference
ABCB1*	Forward: 5′-AGCAGAGGCCGCTGTTCGTT-3′	
	Reverse: 5′-CCATTCCGACCTCGCGCTCC-3′	
ABCG2*	Forward: 5′-TGGAATCCAGAACAGAGCTGGGGT-3′	
	Reverse: 5′-AGAGTTCCACGGCTGAAACACTGC-3′	
TLR-3†	Forward: 5′-TTACGAAGAGGCTGGAATGG-3′	
	Reverse: 5′-AGGAACTCCTTTGCCTTGGT-3′	
TLR-4†	Forward: 5′-ATTTGTCTCCACAGCCACCA-3′	
	Reverse: 5′-ACAGGAAACCCCATCCAGAG-3′	
CCL2*	Forward: 5′-TTCATTCCCCAAGGGCTCGCTCA-3′	
	Reverse: 5′-AGCACAGATCTCCTTGGCCACAA-3′	
IL-6	Forward: 5′-TTGTCAAGACATGCCAAAGTGCT-3′	24
	Reverse: 5′-GCCTCAGACATCTCCAGTCC-3′	
IL-8*	Forward: 5′-GCAGCCTTCCTGATTTCTGCAGCT-3′	
	Reverse: 5′-CCTTGGGGTCCAGACAGAGCTCT-3′	
SDHA	Forward: 5′-TGGGAACAAGAGGGCATCTG-3′	25
	Reverse: 5′-CCACCACTGCATCAAATTCATG-3′	
YWHAZ	Forward: 5′-ACTTTTGGTACATTGTGGCTTCAA-3′	25
	Reverse: 5′-CCGCCAGGACAAACCAGTAT-3′	
CYC1	Forward: 5′-CAGATAGCCAAGGATGTGTG-3′	25
	Reverse: 5′-CATCATCAACATCTTGAGCC-3′	
TOP1	Forward: 5′-GATGAACCTGAAGATGATGGC-3′	25
	Reverse: 5′-TCAGCATCATCCTCATCTCG-3′	

*Gene specific primers were designed with Primer-BLAST (*http://www.ncbi.nlm.nih.gov/tools/primer-blast*).
†Program primers 3-In silico PCR.

Evidence also indicates that the expression and function of these transporters may be disrupted by infection and inflammation.[3,12] This is important because infection and inflammation during pregnancy are associated with complications of pregnancy such as preterm birth and fetal brain damage.[13] In rodents, bacterial exposure and proinflammatory cytokines alter Abcb1a mRNA expression and function in the placenta and other tissues.[12,14,15] Viral infection decreases expression of Abcb1a, Abcb1b, and Abcg2 in the rat placenta but increases expression in the liver.[16] These data suggest that infective agents alter multidrug resistance in a tissue- and infective agent-dependent manner and highlight the need for further studies to investigate how inflammatory mediators, through their actions on the multidrug resistance proteins, might expose the conceptus to potential harmful substances in the maternal circulation.

To date no studies have examined the effect of lipopolysaccharide (LPS; modeling bacterial infection) or polyinosinic–polycytidylic acid (poly(I:C); modeling viral infection) on P-gp and/or BCRP in the human placenta in early and late gestation. However, their respective receptors, Toll-like receptor (TLR)-4 and TLR-3, are expressed in the placenta at term and preterm.[17–19] Therefore, our aim was to examine the effect of LPS and poly(I:C) on placental P-gp and BCRP expressions in the first and third trimesters of human pregnancy by using placental villous cultures, which maintain tissue integrity during culture. We hypothesized that bacterial or viral infection would reduce placental expression of the multidrug transporters P-gp and BCRP in a gestational age-dependent fashion.

Materials and Methods

Placental Tissue Collection and Ethical Approval

Placental specimens were collected by the Research Centre for Women's and Infants' Health BioBank program of the Mount Sinai Hospital after informed consent and in adherence with the policies of Mount Sinai Hospital and the University of Toronto Research Ethic Boards. First-trimester tissues were obtained at 8 to 10 weeks' gestation from patients undergoing surgical termination of pregnancy and at >37 weeks' gestation from term elective cesarean deliveries.

Placental Villous Explants

Placental villous explants were cultured as described previously,[4,20,21] with minor alterations. Briefly, placental specimens were placed into 1% phosphate-buffered saline (PBS) with $Ca^{2+}$and Mg^{2+} and transported to the laboratory. Tissues were dissected into villous clusters of approximately 15 to 30 mg, and three villous explants were cultured per well in 12-well plates that contained Dulbecco's modified Eagle's medium/F12, Normocin antibiotic (Invivogen, San Diego, CA), and 1× insulin, transferrin, and selenium-A (Invitrogen, Grand Island, NY) that was previously equilibrated at 8% O_2 (CO_2, 37°C) for 24 hours. Explants were cultured for 24 hours and then randomly divided into treatment groups. Explants were treated with the TLR-4 ligand, LPS from *Escherichia coli* (0.1, 1, 10 μg/mL[21–23]; Sigma-Aldrich, St. Louis, MO), or the TLR-3