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Impact of endotoxin on the expression of drug transporters in the placenta of HIV-1 transgenic (HIV-Tg) rats

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ABSTRACT

Background: Inflammatory responses in HIV (+) patients may be exacerbated due to reports of subclinical endotoxemia and existing immune dysregulation. As inflammation has been reported to mediate changes in the expression of transporters, this could be potentiated in pregnant HIV (+) women. Similar to humans, the HIV-Tg rat model develops immune dysregulation and chronic AIDS-like conditions. Our objective was to examine the expression of placental drug transporters in HIV-Tg rats in response to low-dose endotoxin.
Methods: Pregnant HIV-Tg rats or wild-type littermates (WT) were treated with low dose bacterial endotoxin 0.1 mg/kg ($n = 8$) or 0.25 mg/kg ($n = 4$–6) on GD18 and placentas were harvested 18 h later. Placental and hepatic expression of transporters and cytokines were examined using qRT-PCR and Western blotting.
Results: As compared to WT, endotoxin administration increased the hepatic and placental expression of IL-6 and TNF-α to a greater extent in HIV-Tg rats ($p < 0.05$). The placental mRNA and protein expression of *Abcb1a* and *Slco2b1* was significantly decreased in endotoxin-treated HIV-Tg but not WT rats and downregulation of *Slco4a1* mRNA was more pronounced in the HIV-Tg group ($p < 0.05$). These changes significantly correlated with the placental expression of pro-inflammatory cytokines. *Abcc3* mRNA expression was increased in the placenta of endotoxin treated WT rats only, while placental expression of *Abcc1*, *Abcc2* and *Abcc4* was not significantly affected in both WT and HIV rats. Endotoxin imposed a pronounced downregulation in the hepatic expression of *Abcb1a, Abcc2, Abcc4, Abcg2, Slco1a1*, *Slco1b2* and *Slc29a1* in both HIV-Tg and WT rats; however, *Abcb1b* expression was increased in HIV but not WT rats.
Conclusion: Our results indicate that low-dose endotoxin resulted in an augmented inflammatory response in HIV-Tg rats accompanied with significant changes in the placental expression of several drug transporters. Our data suggests that subclinical endotoxemia and other co-existing infections may alter the placental transfer of drugs in the HIV population.

1. Introduction

Current treatment guidelines for HIV infection recommend the use of highly active anti-retroviral therapy (HAART) in all HIV infected individuals (AIDsinfo, 2016). In pregnant HIV women; therapy aims at treating maternal HIV infection as well as preventing vertical transmission to the fetus (AIDsinfo, 2014). The success of this therapy has restricted the incidence of vertical transmission to <2% (Cooper et al., 2002; Townsend et al., 2008; Chou et al., 2012), however it has also been associated with acute and chronic drug toxicity (Spector, 2001; Powis et al., 2011). Current treatment guidelines for HIV infected women represent uncomplicated pregnancies, and although HIV infected pregnant women commonly present with secondary infections, this is seldom accounted for in designing dosage regimens. Subclinical endotoxemia commonly occurs in HIV infected individuals (Brenchley et al., 2006; Sandler and Douek, 2012), and has more recently been recognized in pregnant HIV women (Lopez et al., 2016). Microbial translocation from the gut is now considered a key driving force of immune activation and numerous markers of gut mucosal dysfunction remain elevated despite initiation of antiretroviral therapy (Mehandru et al., 2006; Jiang et al., 2009; d'Ettorre et al., 2011; Jenabian et al., 2015). Binding of bacterial lipopolysaccharides (LPS) to toll like receptor 4 (TLR-4), initiates a signaling cascade which results in the release of numerous cytokines including interleukin (IL)-6, IL-1β and tumor necrosis factor (TNF)-α (Lu et al., 2008). This is of particular relevance as elevations in pro-inflammatory cytokines have been shown to alter the expression and function of drug transporters. In human placenta, inflammation was found to downregulate the expression of placental SLCO2B1 and ABCG2 (also known as BCRP) in placentas obtained from women with chorioamnionitis (Petrovic et al., 2015). Another study reported increased expression of P-glycoprotein (P-gp encoded by *ABCB1* in humans and *Abcb1a* and *Abcb1b* in rodents) and ABCG2 in placentas

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isolated from infected women (Mason et al., 2011). Moreover, endotoxin - induced inflammation has been shown to downregulate the placental expression of *Abcb1a* and *Abcg2* in rodents and this was associated with increased fetal accumulation of their substrates (Wang et al., 2005; Petrovic et al., 2008).

Placental drug transporters including the ATP-binding cassette (ABC) and solute carrier (SLC) superfamilies of transporters perform extrusion and uptake functions and are major determinants of fetal drug exposure (Behravan and Piquette-Miller, 2007). ABC efflux drug transporters including ABCB1, ABCG2 and members of the ABCC family prevent trans-placental passage of clinically important drugs including many administered during HAART. Indeed, the protective role of placental drug transporters has supported the use of protease inhibitors in pregnant HIV positive women as a corner stone in HAART (Gulati and Gerk, 2009; Mirochnick and Best, 2010). Although the placental transfer of protease inhibitors is considered minimal, it is highly variable. For example, cord to maternal blood ratios of lopinavir have been reported to range from 5 to 25% and that of nelfinavir from 7 to 74% (van Heeswijk et al., 2004; Gingelmaier et al., 2006). It is plausible that co-existing maternal conditions including infections could have contributed to this variability at least partially due to disease-mediated changes in placental drug transporters.

HIV infection has been reported to alter the expression of ABC drug transporters in peripheral blood mononuclear cells and the rectal-sigmoid colon of HIV infected patients as compared to healthy volunteers (Meaden et al., 2001; De Rosa et al., 2013). It is proposed that HIV viral proteins play a significant role in altering the expression of drug transporters. This has been demonstrated mainly through *in vitro* studies showing decreased expression and function of P-gp in rat and human brain astrocytes incubated with the HIV viral protein gp120, whereby effects were mediated *via* increased pro-inflammatory cytokines IL-6, IL-1β and TNF-α (Ronaldson and Bendayan, 2006; Ashraf et al., 2011). Further *in vivo* studies in the HIV transgenic rat (HIV-Tg) demonstrating altered expression of P-gp mRNA transcripts support these *in vitro* findings (Robillard et al., 2014). It has been reported that several HIV viral proteins including gp120, tat and nef are capable of activating lymphocytes and macrophages, causing the release of pro-inflammatory cytokines IL-6 and TNF-α (Lee et al., 2003; Swingler et al., 2003; Appay and Sauce, 2008; Yim et al., 2009; Mogensen et al., 2010).

Altered expression of placental drug transporters has been linked to higher transplacental passage, as well as drug-induced birth defects in rodents (Lankas et al., 1997; Smit et al., 1999; Choo et al., 2000; Pavek et al., 2003). Given the high incidence of subclinical endotoxemia in HIV infected individuals, our objective was to examine the impact of low-dose endotoxin on the expression of placental drug transporters in a pregnant HIV-Tg rat model. The HIV-Tg rat is a convenient *in vivo* model that demonstrates effects of HIV viral proteins in the absence of viral replication (Reid et al., 2001). The model also shares similarity with immune dysregulation observed in HIV infected individuals (Chang et al., 2007b; Peng et al., 2010; Abbondanzo and Chang, 2014). A downregulation of several placental transporters has been previously reported in endotoxin-treated rats (Petrovic et al., 2008). We thus hypothesized that the response to endotoxin would be potentiated in the pregnant HIV-Tg model resulting in further downregulation in the expression of placental drug transporters than would be seen with endotoxin or HIV alone.

2. Material and methods

2.1. Animals and experimental design

HIV-Transgenic and F344/NHsd rats, purchased from Harlan (Indianapolis, IN) were bred and female HIV-Tg and wild-type (WT) littermates were separated at weaning and mated with WT males at 3–4 months of age. Pregnant HIV-Tg rats or WT were treated intraperitoneally with a low dose of LPS 0.1 mg/kg ($n = 8$/group) or 0.25 mg/kg ($n = 4$ HIV-Tg, $n = 6$ WT) (*E. coli* 055:B5, Sigma-Aldrich, Oakville, ON) or saline on gestational day 18 ($n = 5$ HIV-Tg, $n = 7$ WT). Placentas, livers and fetal tails were harvested 18 h later and immediately frozen in liquid nitrogen and stored at −80 °C for further analysis. All animal studies were approved by the University of Toronto Animal Care Committee and the Canadian Council on Animal Care.

Amplification of HIV viral protein gp120 using fetal tails was used to determine fetal genotype within the HIV-Tg dam. PCR products were separated by gel electrophoresis on a 2% agarose gel as previously described (Ghoneim and Piquette-Miller, 2016).

2.2. Serum cytokine analysis

Cytokine concentrations were measured in serum using commercially available rat specific ELISA kits (R&D Systems, Minneapolis, MN). The limit of detection (LD) for IL-6, IL-1β, TNF-α and IFN-γ were 36, 5, 9 and 10 pg/ml, respectively.

2.3. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

The mRNA levels were determined using qRT-PCR as previously described (Ghoneim and Piquette-Miller, 2016). Briefly, RNA was extracted using the TRIzol extraction method (Invitrogen, Carlsbad, CA) according to manufacturer instructions and reverse transcribed (RT) with High Capacity cDNA RT kit (Applied Biosystems, Foster City, CA). Real-time quantitative PCR was performed using Power SYBR Green detection system (ABI HT 7900, Applied Biosystems, Streetsville, ON, Canada). To calculate relative mRNA expression, comparative Ct (ΔΔCt) method was used and each gene of interest was normalized to the house keeping gene β-actin. Similar results were obtained using β-actin and GAPDH. Specific primer sequences have been previously reported (Ghoneim and Piquette-Miller, 2016).

2.4. Western blot analysis

Protein was extracted from placental tissue as previously described (Anger et al., 2012; Ghoneim et al., 2015). Briefly, 160 mg of tissue was homogenized using 1× RIPA lysis buffer (Cell Signalling Technology, Denver, USA) with freshly added phenylmethylsulfonyl fluoride 0.5 mM (Bioshop, Burlington, ON, Canada) and 4 μl/ml protease inhibitor P8340 (Sigma-Aldrich). Following centrifugation (18,000*g*, 15 min) the supernatant was quantified by Bradford assay. 60 μg protein samples in Laemmli sample buffer (Biorad, Hercules, CA, USA) were separated on a 8% SDS PAGE gel and transferred to polyvinylidene fluoride membranes (Bio-Rad Laboratories Canada, Ltd., Mississauga, ON, Canada). Membranes were blocked in 5% w/v skimmed milk in TBST and incubated over night with anti-P-gp C219 1:200 (Enzo Life Sciences, Farmingdale, NY) and anti-SLCO2B1 1:1000 (Sigma-Aldrich, Oakville, ON). Following a series of washes, membranes were incubated with secondary antibodies (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) anti-mouse 1:3000 and anti-rabbit 1:10,000. Immunodetectable protein was quantified using Alpha Ease FC imaging software (Alpha Innotech). To control for loading variability β-actin AC-15 1:10,000 (Sigma-Aldrich, Oakville, ON) was used in addition to the use of a calibrator sample to control for different gels.

2.5. Statistical analysis

Analysis was performed using PRISM 6 software. Two-way ANOVA followed by Fisher's LSD was used to determine effect of HIV and LPS. Unpaired student *t*-test was used to compare differences between HIV-Tg and WT pups within the HIV-Tg dams as well physiological changes between HIV-Tg and WT dams.