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Toxicology 211 (2005) 242–252
TOXICOLOGY
www.elsevier.com/locate/toxicol

Lipopolysaccharide treatment downregulates the expression of the *pregnane X receptor*, *cyp3a11* and *mdr1a* genes in mouse placenta

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Received 25 February 2005; received in revised form 19 March 2005; accepted 23 March 2005

Abstract

The *cytochrome P450 3A* (*CYP3A*) is a member of the cytochrome P450 monooxygenase superfamily. The *multidrug resistance 1* (*MDR1*) gene belongs to the ATP-binding cassette (ABC) family. *Pregnane X receptor* (*PXR*) is a nuclear receptor that regulates its target gene transcription in a ligand-dependent manner. Lipopolysaccharide (LPS)-induced downregulation of PXR, CYP3A and MDR1 in liver has been demonstrated in a series of studies. However, it is not clear whether LPS represses the expression of *PXR*, *CYP3A* and *MDR1* in placenta. In the present study, we investigated the effects of LPS on the expression of *PXR*, c*yp3a11* and *mdr1a* in mouse placenta. Pregnant ICR mice were injected intraperitoneally with different doses of LPS (0.1–0.5 mg/kg) on gestational day (gd) 17. Placental *PXR*, *cyp3a11* and *mdr1a* mRNA levels were determined at 12 h after LPS treatment using RT-PCR. Results showed that LPS significantly downregulated *PXR*, *cyp3a11* and *mdr1a* mRNA levels in a dose-dependent manner. LPS-induced downregulation of *PXR*, *cyp3a11* and *mdr1a* mRNA in placenta was significantly attenuated after pregnant mice were pre- and post-treated with alpha-phenyl-*N*-*t*-butylnitrone (PBN), a free radical spin trapping agent. Additional experiments revealed that LPS increased *lipid peroxidation* and *proinflammatory cytokine expressions* in mouse placenta, all of which were also attenuated by PBN. Furthermore, LPS-induced downregulation of *PXR*, *cyp3a11* and *mdr1a* mRNA in mouse placenta was prevented by *N*-acetylcysteine (NAC). NAC also inhibited LPS-initiated *lipid peroxidation*, *GSH depletion* and *proinflammatory cytokine expressions* in mouse placenta. These results indicated that LPS downregulates placental *PXR*, *cyp3a11* and *mdr1a*

Abbreviations: cDNA, complementary DNA; CYP3A, cytochrome P450 3A; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-1, interleukin-1; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MDR1, multidrug resistance 1; NAC, *N*-acetylcysteine; NF-kB, nuclear factor-kB; NO, nitric oxide; O^{2-}, superoxide anion; PBN, alpha-phenyl-*N*-*t*-butylnitrone; PXR, pregnane X receptor; ROS, reactive oxygen species; RT-PCR, reverse transcription polymerase chain reaction; TNF-α, tumor necrosis factor-α

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0300-483X/$ – see front matter
doi:10.1016/j.tox.2005.03.011

mRNA expressions. Reactive oxygen species (ROS) may be involved in LPS-induced downregulation of *PXR*, *cyp3a11* and *mdr1a* in mouse placenta.

Keywords: Lipopolysaccharide; *Pregnane X receptor*; *Cytochrome P450 3a*; *Multidrug resistance 1*; Reactive oxygen species; Placenta

1. Introduction

The *cytochrome P450 3A* (*CYP3A*) is a member of the cytochrome P450 monooxygenase superfamily. In human, *CYP3A4* and *CYP3A5* gene products account for 30–40% of the total cytochrome P450 in the adult liver, which is responsible for the oxidative metabolism of numerous clinically used drugs and toxicants (Thummel et al., 1998). Although *CYP3A4* and *CYP3A5* in fetal liver are not detectable, fetal hepatocytes express *CYP3A7* as early as 50–60 days gestation with continued significant levels of expression through the perinatal period (Stevens et al., 2003). Expression of CYP3A, an enzyme that catalyzes drugs and xenobiotics, can be detected in human placenta as early as the first trimester of pregnancy (Hakkola et al., 1996a,b). In mice, *cyp3a11* and *cyp3a13* are major members of *cyp3a* subfamily in the adult liver. In the developing mouse embryo, the amount of *cyp3a11* and *cyp3a13* expressions gradually increases with the advancement of embryonic development (Choudhary et al., 2003). *Multidrug resistance 1* (*MDR1*) gene belongs to the ATP-binding cassette (ABC) family. *MDR1* encodes P-glycoprotein (P-gp), which functions as a transmembrane efflux pump that translocates its substrates from its intracellular domain to its extracellular domain (Fromm, 2004). P-glycoprotein is expressed constitutively in small intestine and liver. *Pregnane X receptor* (*PXR*) is a member of the nuclear receptor superfamily, which regulates *CYP3A* and *MDR1* gene transcription in a ligand-dependent manner (Kliewer et al., 1998; Lehmann et al., 1998; Bertilsson et al., 1998; Teng and Piquette-Miller, 2005).

Lipopolysaccharide (LPS) is a toxic component of cell walls of Gram-negative bacteria and is widely present in the digestive tracts of humans and animals. Humans are constantly exposed to low levels of LPS through infection. Gastrointestinal distress and alcohol drinking often increase permeability of LPS from gastrointestinal tract into blood (Mathurin et al., 2000). On the other hand, numerous studies indicated that inflammation and infection reduce hepatic CYP levels in various species including human, rat and mouse (Morgan, 1997, 2001). The effect of LPS on P450 expression is very well documented in a variety of systems and tissues (Renton and Nicholson, 2000; Li-Masters and Morgan, 2001; Morgan et al., 2002; Pan et al., 2003; Kalitsky-Szirtes et al., 2004). LPS-induced downregulation of *cyp3a* in liver has also been demonstrated in mouse model (Sewer et al., 1998). Moreover, LPS-induced downregulation of hepatic CYP3A is associated with a marked reduction in *PXR* mRNA and protein levels (Beigneux et al., 2002; Sachdeva et al., 2003). Our earlier studies showed that reactive oxygen species (ROS) mediate LPS-induced downregulation of *PXR* and its target gene *cyp3a* in mouse liver (Xu et al., 2004, 2005).

On the other hand, *PXR*, *CYP3A* and *MDR1* were also expressed in placenta of human and rodent animals (Masuyama et al., 2001; Leazer and Klaassen, 2003; Novotna et al., 2004). Together with xenobiotic-metabolizing enzymes, *MDR1* encoded P-gp in placenta is a drug efflux transporter that limits the entry of various potentially toxic drugs and xenobiotics into the fetus and is thus considered a placental protective mechanism (Lankas et al., 1998). Several studies have demonstrated that placental P-gp deficiency enhances susceptibility to chemically induced birth defects in mice (Lankas et al., 1998; Smit et al., 1999). However, it is not clear whether LPS represses the expression of *PXR*, *CYP3A* and *MDR1* in placenta.

In present study, we investigated the effects of LPS on *PXR*, *cyp3a11* and *mdr1a* gene expressions in mouse placenta. Our results found that LPS downregulates placental *PXR*, *cyp3a11* and *mdr1a* gene expressions. ROS may be involved in LPS-induced downregulation of *PXR*, *cyp3a11* and *mdr1a* in mouse placenta.