Toxicology Letters 179 (2008) 71–77

Contents lists available at ScienceDirect

Toxicology Letters

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

Tumor necrosis factor alpha partially contributes to lipopolysaccharide-induced downregulation of CYP3A in fetal liver: Its repression by a low dose LPS pretreatment

Xiang-Yun Li[1], Cheng Zhang[1], Hua Wang, Yan-Li Ji, Su-Fang Wang, Lei Zhao, Xi Chen, De-Xiang Xu*

Department of Toxicology, Anhui Medical University, Hefei 230032, China

ARTICLE INFO

Article history:
Received 18 January 2008
Received in revised form 4 April 2008
Accepted 9 April 2008
Available online 22 May 2008

Keywords:
Lipopolysaccharide
Cytochrome P450
Fetal liver
Tumor necrosis factor alpha

ABSTRACT

With embryonic development, fetal hepatocytes gradually express various types of cytochromes P450 (CYPs) that play a key role in the detoxification of xenobiotics. In the present study, we showed that maternal lipopolysaccharide (LPS) exposure downregulated *cyp3a11* mRNA and CYP3A protein in fetal liver. The increased level of TNF-α protein in fetal liver, transferred from either the maternal circulation or amniotic fluid, seems to be associated with LPS-induced downregulation of *cyp3a11* mRNA and CYP3A protein in fetal liver. Interestingly, a low dose LPS (10 μg/kg) pretreatment attenuated LPS-induced downregulation of cyp3a11 mRNA and CYP3A protein in fetal liver. Correspondingly, a low dose LPS pretreatment attenuated LPS-induced downregulation of *pregnane X receptor* (*pxr*) in fetal liver. Additional experiment showed that a low dose LPS pretreatment decreased the level of TNF-α in maternal serum and amniotic fluid and counteracted LPS-induced expression of TNF-α mRNA in maternal liver and placenta. Although a low dose LPS pretreatment alleviated LPS-induced increase in TNF-α in fetal liver, it had little effect on TNF-α mRNA in fetal liver. These results suggest that a low dose LPS pretreatment protects fetuses against LPS-induced downregulation of hepatic *cyp3a11* and *pxr* expression through the repression of maternally sourced TNF-α production.

1. Introduction

The liver is the major organ of amino acid and lipid metabolism, gluconeogenesis, synthesis of serum proteins, and xenobiotic detoxification. The fetal liver functions as the major hematopoietic organ in the mid- to late fetal stage (Dzierzak and Medvinsky, 1995; Hardy and Hayakawa, 2001). With embryonic development, fetal hepatocytes gradually express various types of cytochromes P450 (CYPs) that play a key role in the detoxification of drug or other xenobiotics (Hulla and Juchau, 1989; Krauer and Dayer, 1991; Rich and Boobis, 1997; de Wildt et al., 1999; Hines and McCarver, 2002). CYP3A is a member of the cytochrome P-450 monooxygenase superfamily. In human, CYP3A4 and CYP3A5 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 (Goodwin et al., 2002). Although CYP3A4 and CYP3A5 are not detectable in fetal liver, fetal hepatocytes express CYP3A7 as early as gestational day 50–60 (Stevens et al., 2003). In mice, *cyp3a11* and *cyp3a13* are major members of *cyp3a* subfamily in the adult liver (Stevens et al., 2003; Anakk et al., 2003). In the developing mouse embryo, the amount of *cyp3a11* and *cyp3a13* expression gradually increases with the advancement of embryonic development (Choudhary et al., 2003).

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 (Zhou et al., 2003). Numerous studies have demonstrated that LPS decreases the levels of CYP3A in livers of rats and mice (Morgan et al., 2002; Aitken et al., 2006). On the other hand, maternal LPS exposure results in intra-uterine fetal death and intra-uterine growth retardation in animals (Xu et al., 2005a, 2006, 2007; Chen et al., 2006). Recently, we found that the expression of hepatic *cyp3a11* mRNA was significantly decreased in fetuses from dams that were exposed to LPS during pregnancy (Xu et al., 2005b). In the present study, we investigated the role of TNF-α in LPS-induced

Abbreviations: CYP, cytochrome; ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LPS, lipopolysaccharide; PXR, pregnane X receptor; PTX, pentoxifylline; RT-PCR, reverse transcription polymerase chain reaction; TNF, tumor necrosis factor.

* Corresponding author. Tel.: +86 551 5161170/5167923.
E-mail address: xudex@126.com (D.-X. Xu).

[1] These authors contributed equally to this work.

0378-4274/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.toxlet.2008.04.005

downregulation of CYP3A in fetal liver. Our results indicate that the increased level of TNF-α in fetal liver, possibly sourced from maternal circulation and perhaps from amniotic fluid, contributes, at least partially, to LPS-induced downregulation of CYP3A in fetal liver. A low dose LPS pretreatment protects fetuses against LPS-induced downregulation of CYP3A through inhibiting the release of maternally sourced TNF-α.

2. Materials and methods

2.1. Chemicals

Lipopolysaccharide (*Escherichia coli* LPS, serotype 0127:B8) and pentoxifylline (PTX) were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were from Sigma or as indicated in the specified methods.

2.2. Animals and treatments

The ICR mice (8–10-week-old; male mice: 28–30 g; female mice: 24–26 g) were purchased from Beijing Vital River (Beijing, China). The animals were allowed free access to food and water at all times and were maintained on a 12-h light:12-h dark cycle in a controlled temperature (20–25 °C) and humidity (50 ± 5%) environment for a period of 1 week before use. For mating purposes, four females were housed overnight with two males starting at 9:00 p.m. Females were checked by 7:00 a.m. the next morning, and the presence of a vaginal plug was designated as gestational day (gd) 0.

To investigate the effects of maternal LPS exposure on CYP3A in fetal liver, the pregnant mice were intraperitoneally (i.p.) injected with LPS (either 0.2 mg/kg or 0.5 mg/kg) on gd 17. To investigate the effects of PTX on LPS-induced downregulation of CYP3A in fetal liver, the pregnant mice were i.p. injected with 0.5 mg/kg LPS on gd 17. In PTX treatment group, the pregnant mice were injected with PTX (100 mg/kg, i.p.) 30 min prior to LPS. The pregnant mice were sacrificed 12 h after LPS. Fetal livers were excised for reverse transcription polymerase chain reaction (RT-PCR) and western blotting. To investigate the effects of a low dose LPS pretreatment on LPS-induced downregulation of *cyp3a11* mRNA and CYP3A protein in fetal liver, the pregnant mice were pretreated with a low dose LPS (10 μg/kg, i.p.) 24 h prior to high-dose LPS (0.5 mg/kg, i.p.). Twelve pregnant mice each group were sacrificed 1.5 h after LPS for measurement of TNF-α. The remaining pregnant mice were sacrificed 12 h after LPS. Fetal livers were excised for RT-PCR and western blotting. All procedures on animals followed the guidelines for humane treatment set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University.

2.3. Reverse transcription polymerase chain reaction

Total cellular RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RT and PCR were performed as described previously (Chen et al., 2005). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal positive control standard. Following primers were synthesized by Sangon Biological Technology (Shanghai, China). GAPDH, 5′-GAG GGG CCA TCC ACA GTC TTc-3′ and 5′-CAT CAC CAT CTT CCA GGA GCG-3′; pregnane X receptor (PXR), 5′-GCG CGG AGA AGA CGG CAG CAT C-3′ and 5′-CCC AGG TTC CCG TTT CCG TGT C-3′; Cyp3a11, 5′-CTC AAT GGT GTG TAT ATC CCC-3′ and 5′-CCG ATG TTC TTA GAC ACT GCC-3′; TNF-α, 5′-GGC AGG TCT ACT TTG GAG TCA TTG C-3′ and 5′-ACA TTC GAG GCT CCA GTG AAT TCG G-3′. The amplified PCR products were electrophored at 75 V through 1.5% agarose gels (Sigma) for 45 min. The pBR322 DNA digested with AluI was used for molecular markers (MBI Fermentas). Agarose gels were stained with 0.5 μg/ml ethidium bromide (Sigma) TBE buffer.

2.4. Enzyme-linked immunosorbent assay (ELISA)

The fetal liver tissue (100 mg) was placed in 1 ml of iced lysis buffer containing 50 mM Tris–HCl, 150 mM NaCl, 1% NP-40, 0.25% Na-deoxycholate, 1 mM EDTA, and 0.1 mM phenylmethylsulphonyl fluoride (PMSF) (recipe from Upstate, Charlottesville, VA). Samples were homogenized and centrifuged at 12,000 rpm for 20 min at 4 °C. Supernatants were aliquoted for measurement of TNF-α, IL-1β and IL-6. Commercial ELISA kits (R&D Systems) were used to determine level of TNF-α according to the manufacturer's protocol.

2.5. Western blot analysis

Samples from fetal liver were homogenized in lysis buffer containing 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecylsulphate (SDS), and 1 mM phenylmethylsulphonyl fluoride. Samples were then centrifuged at 15,000 × *g* for 15 min. Supernatants from each sample were added to a gel loading buffer (100 mM Tris, pH 6.8, 20% glycerol, 200 mM DTT, 4% SDS, 0.03% bromophenol blue) and boiled for 5 min. Proteins (50 μg/sample) in loading buffer were subjected to electrophoresis in 10% SDS–polyacrylamide gel for 3 h. The gel was transferred electrophoretically onto a polyvinylidene fluoride membrane (Immobilon-P; Millipore Corp., Bedford, MA, USA) and blocked in 5% nonfat powdered milk in Dulbecco's PBS (DPBS) overnight at 4 °C. The membranes were then incubated for 2 h with rabbit polyclonal antibody against mouse CYP3A (Alexis Biochemicals, USA) (1:1000 dilutions) or β-actin (Beijing Biosynthesis Biotechnology Inc., Beijing, China) (1:2000 dilutions) at room temperature. After washes in DPBS containing 0.05% Tween-20 four times for 10 min each, the membranes were incubated with goat anti-rabbit IgG antibody for 2 h. The membranes were then washed for four times in DPBS containing 0.05% Tween-20 for 10 min each, followed by signal development using an enhanced chemiluminescence (ECL) detection kit from Pierce (Pierce Biotechnology, Inc., Rockford, IL).

2.6. Statistical analysis

For western blot studies, CYP3A were normalized to β-actin level in the same samples. The densitometry unite of the control was assigned as 1. For analyzing the ontogeny of CYP3A in fetal liver, the densitometry unite of adult liver was assigned as 1. For RT-PCR, cyp3a11, PXR and TNF-α mRNA levels were normalized to GAPDH mRNA level in the same samples. Cyp3a11, PXR and TNF-α mRNA level of the control was assigned as 100%. Quantified data were expressed as means ± S.E. at each point. ANOVA and the Student–Newmann–Keuls post hoc test were used to determine differences among different groups.

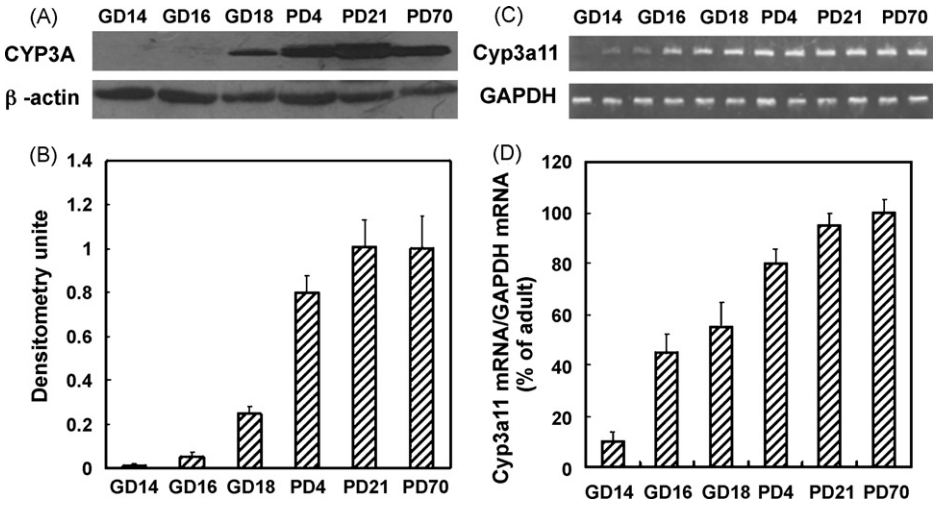

Fig. 1. Ontogeny of hepatic cyp3a11 mRNA and CYP3A protein in fetal, neonatal and adult mice. (A) Immunoreactive CYP3A (upper panel) and β-actin (lower panel). (B) Quantitative analysis of scanning densitometry on four samples at each time point was performed. (C) Cyp3a11 (upper panel) and GAPDH (lower panel) mRNA in fetal liver. (D) Quantitative analysis of scanning densitometry on six samples at each time point was performed. All data were expressed as means ± S.E.

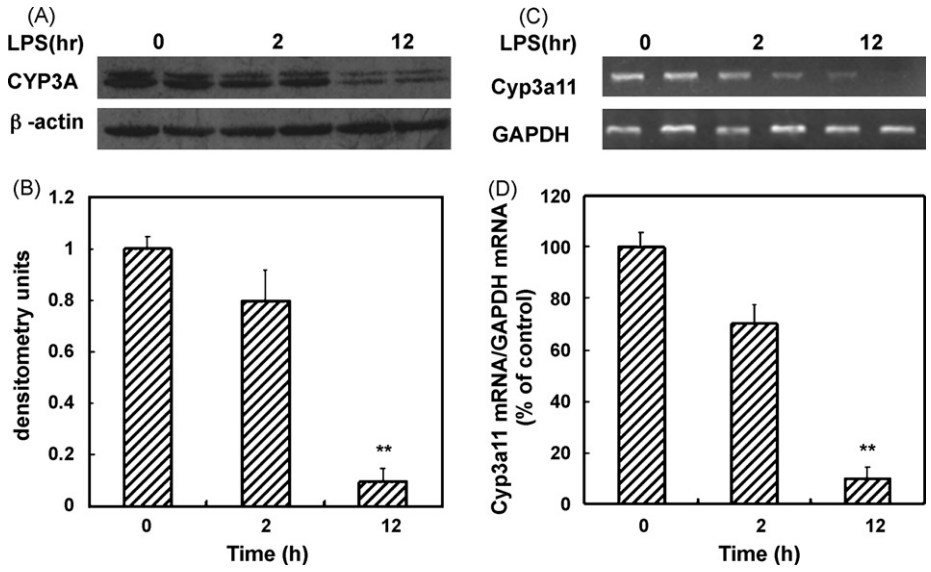

Fig. 2. The effects of maternal LPS exposure on cyp3a11 mRNA and CYP3A protein in fetal liver—the time-course analysis. The pregnant mice were injected with a single dose of LPS (0.5 mg/kg, i.p.) on gd 17. Fetal livers were excised at 2 h or 12 h after LPS treatment. (A) Immunoreactive CYP3A (upper panel) and β-actin (lower panel). (B) Quantitative analysis of scanning densitometry on four samples at each time point was performed. (C) Cyp3a11 (upper panel) and GAPDH (lower panel) mRNA in fetal liver. (D) Quantitative analysis of scanning densitometry on six samples at each time point was performed. All data were expressed as means ± S.E. $^{**}P < 0.01$ as compared with control group.

3. Results

3.1. Ontogeny of CYP3A in fetal liver

The ontogeny of cyp3a11 mRNA and CYP3A protein in mouse fetal liver is presented in Fig. 1. The level of cyp3a11 mRNA and CYP3A protein was very low in fetal liver from gd 14 to 16. On gd 18, the expression level of CYP3A in fetal liver was about 25% of that in adult liver, whereas the level of cyp3a11 mRNA in fetal liver was about 55% of that in adult liver. The level of cyp3a11 mRNA and CYP3A protein was greatly increased after birth. On postnatal day 4, the level of cyp3a11 mRNA and CYP3A protein was about 80% of that in adult liver.

3.2. Maternal LPS exposure downregulates CYP3A in fetal liver

To investigate the effects of maternal LPS exposure on the expression of CYP3A in fetal liver, the pregnant mice were i.p. injected with 0.5 mg/kg LPS on gd 17. Results showed that 0.5 mg/kg LPS administered to the pregnant mice on gd 17 did not cause preterm labor and fetal death within 12 h. The expression of cyp3a11 mRNA and CYP3A protein in fetal liver was almost completely inhibited 12 h after LPS, whereas no significant difference in the level of cyp3a11 mRNA and CYP3A protein in fetal liver was observed 2 h after maternal LPS exposure (Fig. 2). To explore whether a dose–response relationship existed, the pregnant mice were i.p. injected with different doses of LPS (0.2 mg/kg

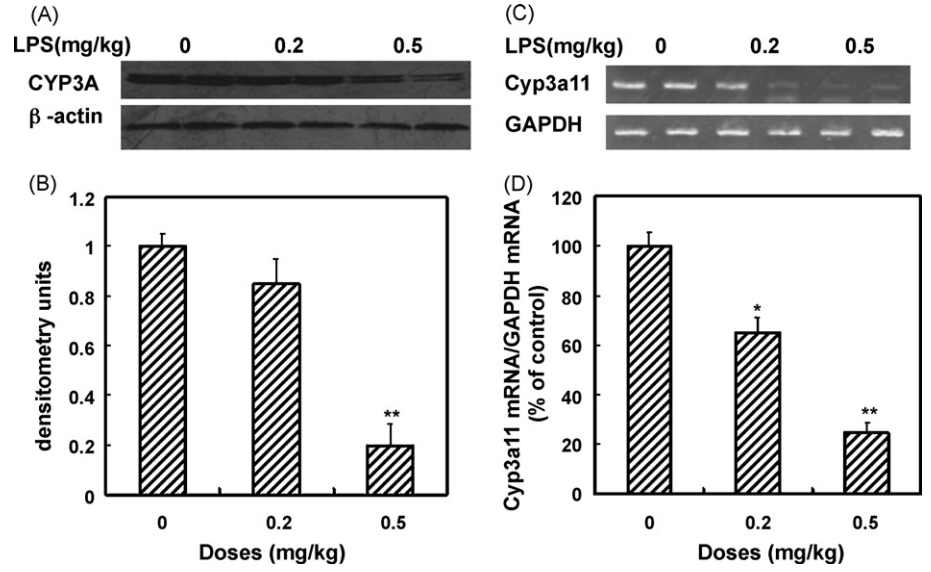

Fig. 3. The effects of maternal LPS exposure on CYP3A in fetal liver—the dose–effect relationship. The pregnant mice were injected with different doses of LPS (0.2 mg/kg or 0.5 mg/kg, i.p.) on gd 17. Fetal livers were excised at 12 h after LPS treatment. (A) Immunoreactive CYP3A (upper panel) and β-actin (lower panel). (B) Quantitative analysis of scanning densitometry on four samples at each time point was performed. (C) Cyp3a11 (upper panel) and GAPDH (lower panel) mRNA in fetal liver. (D) Quantitative analysis of scanning densitometry on six samples at each time point was performed. All data were expressed as means ± S.E. $^{**}P < 0.01$ as compared with control group.

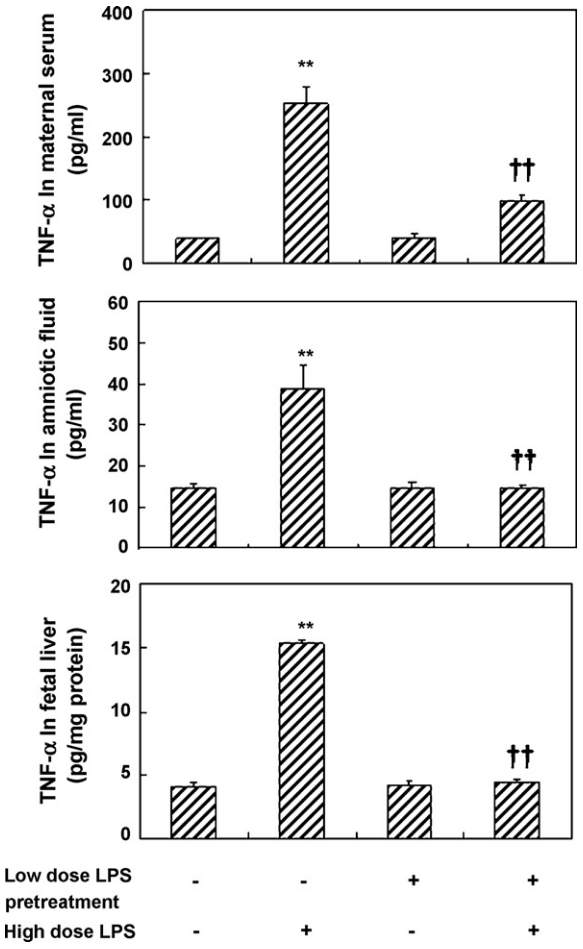

Fig. 4. The effects of maternal LPS exposure on TNF-α in maternal serum, amniotic fluid and fetal liver. The pregnant mice were injected with 0.5 mg/kg LPS (i.p.) on gd 17. Some pregnant mice were pretreated with a low dose LPS (10 μg/kg, i.p.) 24 h before high-dose LPS. TNF-α was measured in maternal serum, amniotic fluid and fetal liver 1.5 h after high-dose LPS. Data were expressed as means ± S.E. ($n = 12$). $^{**}P < 0.01$ as compared with control group. $^{††}P < 0.01$ as compared with LPS group.

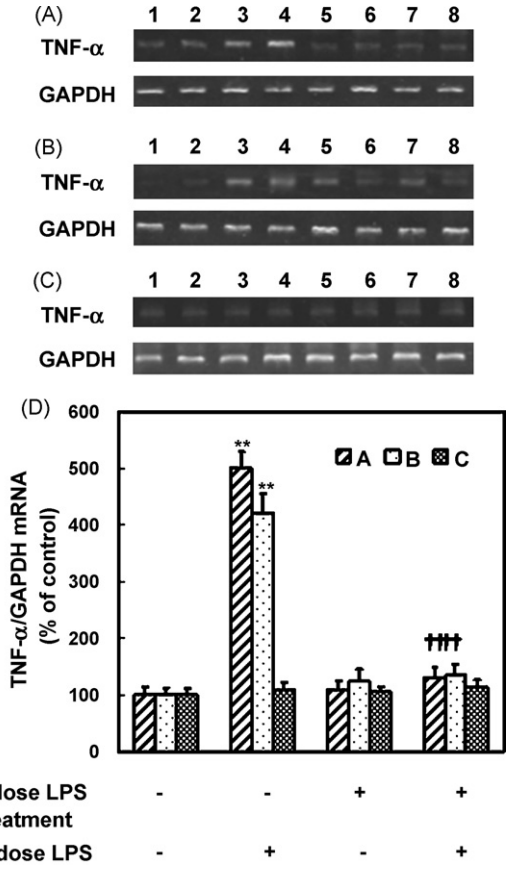

Fig. 5. The effects of maternal LPS exposure on the expression of TNF-α mRNA in maternal liver, placenta and fetal liver. The pregnant mice were injected with a single dose of LPS (0.5 mg/kg, i.p.) on gd 17. Some pregnant mice were pretreated with a low dose LPS (10 μg/kg, i.p.) 24 h before a high-dose LPS. TNF-α mRNA in maternal liver, placenta and fetal liver was measured at 1.5 h after high-dose LPS treatment. TNF-α (upper panel) and GAPDH (lower panel) mRNA in (A) maternal liver, (B) placenta and (C) fetal liver. (1) and (2) Saline; (3) and (4) high-dose LPS; (5) and (6) low-dose LPS pretreatment; (7) and (8) low-dose LPS pretreatment + high-dose LPS. (D) The TNF-α mRNA was normalized to GAPDH mRNA level in the same samples. The TNF-α mRNA level of the control was assigned as 100%. Data were expressed as means ± S.E. ($n = 6$). $^{**}P < 0.01$ as compared with control group. $^{††}P < 0.01$ as compared with LPS group.

or 0.5 mg/kg) on gd 17. As shown in Fig. 3, LPS-induced downregulation of cyp3a11 mRNA and CYP3A protein in fetal liver was dose-dependent.

3.3. Maternal LPS exposure stimulates the release of TNF-α

As shown in Fig. 4, maternal LPS exposure significantly increased the level of TNF-α protein in maternal serum (38.58 ± 1.28 pg/ml vs. 252.62 ± 25.82 pg/ml) and amniotic fluid (14.72 ± 0.87 pg/ml vs. 38.73 ± 5.93 pg/ml). Interestingly, maternal LPS exposure also significantly increased the level of TNF-α in fetal liver (4.17 ± 0.31 pg/mg protein vs. 15.29 ± 0.36 pg/mg protein, $P < 0.01$). The effects of LPS on TNF-α mRNA are presented in Fig. 5. Results showed that maternal LPS exposure significantly upregulated the expression of TNF-α mRNA in maternal liver and placenta. However, no significant difference in TNF-α mRNA level was observed in fetal liver among different groups. The present study showed that a low dose LPS pretreatment significantly attenuated LPS-evoked release of TNF-α in maternal serum, amniotic fluid and fetal liver (Fig. 4). Moreover, a low dose LPS pretreatment counteracted LPS-induced upregulation of TNF-α mRNA in maternal liver and placenta, although it did not affect the expression of TNF-α mRNA in fetal liver (Fig. 5).

3.4. PTX pretreatment attenuates LPS-induced downregulation of cyp3a11 mRNA and CYP3A protein in fetal liver

PTX is an inhibitor of TNF-α synthesis. To explore whether TNF-α is involved in maternal LPS-induced downregulation of CYP3A in fetal liver, the pregnant mice were i.p. injected with PTX (100 mg/kg) 30 min prior to LPS. Results showed that PTX alone did not affect the expression of CYP3A in fetal liver. Interestingly, PTX pretreatment significantly attenuated LPS-induced downregulation of cyp3a11 mRNA and CYP3A protein in fetal liver (Fig. 6).

3.5. A low dose LPS pretreatment protects fetuses against LPS-induced downregulation of CYP3A in fetal liver

The effects of a low dose LPS pretreatment on LPS-induced downregulation of cyp3a11 mRNA and CYP3A protein in fetal liver are analyzed. As shown in Fig. 7, LPS-induced downregulation of cyp3a11 mRNA and CYP3A protein in fetal liver was significantly attenuated by a low dose LPS pretreatment. Our earlier study showed that LPS-induced downregulation of cyp3a11 mRNA in fetal liver was associated with the decreased expression of pregnane X

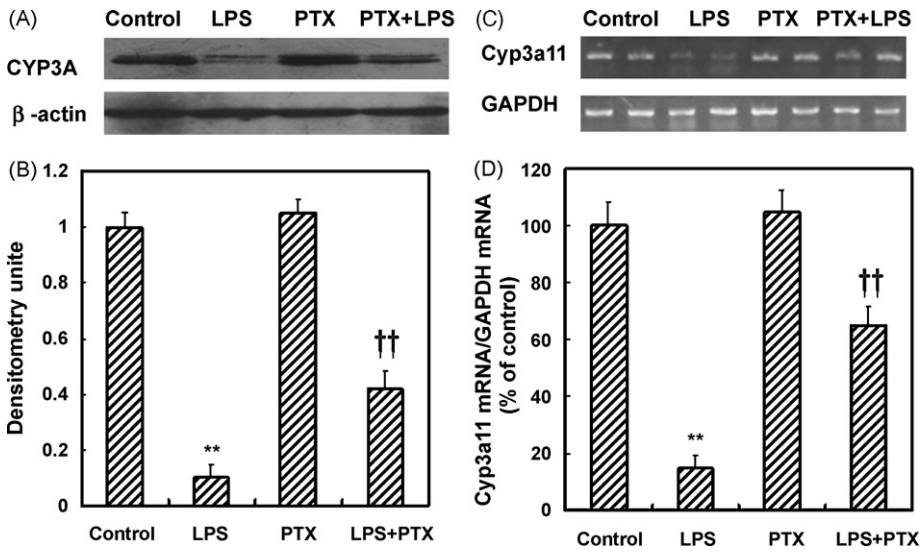

Fig. 6. The effects of PTX on LPS-induced downregulation of CYP3A in fetal liver. The pregnant mice were injected with LPS (0.5 mg/kg, i.p.) on gd 17. Some pregnant mice were injected with PTX (100 mg/kg, i.p.) 30 min before LPS. Fetal livers were excised at 12 h after LPS. (A) Immunoreactive CYP3A (upper panel) and β-actin (lower panel). (B) Quantitative analysis of scanning densitometry on four samples at each time point was performed. (C) Cyp3a11 (upper panel) and GAPDH (lower panel) mRNA in fetal liver. (D) Quantitative analysis of scanning densitometry on six samples at each time point was performed. All data were expressed as means ± S.E. $^{**}P < 0.01$ as compared with control group. $^{††}P < 0.01$ as compared with LPS group.

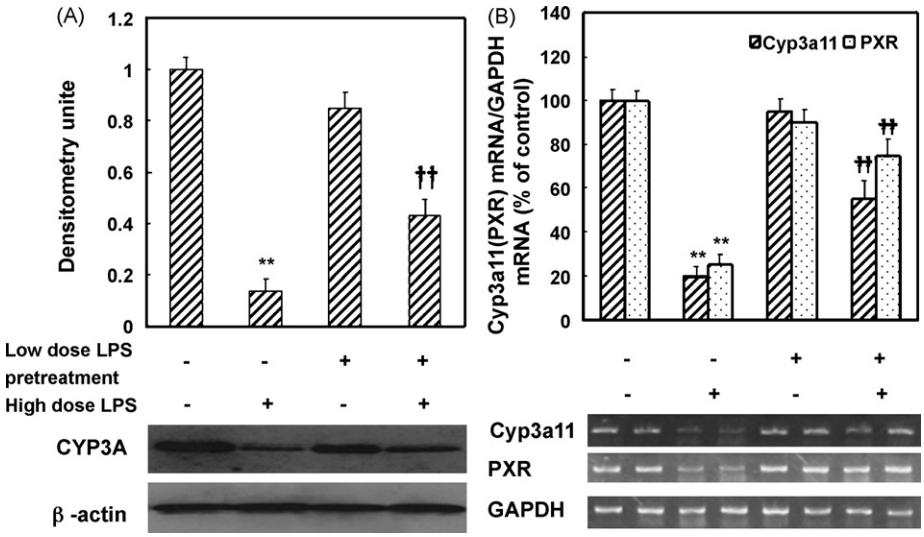

Fig. 7. The effects of a low dose LPS pretreatment on LPS-induced downregulation of CYP3A in fetal liver. The pregnant mice were injected with LPS (0.5 mg/kg, i.p.) on gd 17. Some pregnant mice were pretreated with a low dose of LPS (10 μg/kg, i.p.) 24 h before high-dose LPS (0.5 mg/kg, i.p.). Fetal livers were excised at 12 h after high-dose LPS. (A) Immunoreactive CYP3A (upper panel) and β-actin (lower panel). (B) Quantitative analysis of scanning densitometry on four samples at each time point was performed. (C) Cyp3a11 (upper panel), PXR (middle panel) and GAPDH mRNA (lower panel) in fetal liver. (D) Quantitative analysis of scanning densitometry on six samples at each time point was performed. Data were expressed as means ± S.E. $^{**}P < 0.01$ as compared with control group. $^{††}P < 0.01$ as compared with LPS group.

receptor mRNA in fetal liver (Xu et al., 2005b). Therefore, we analyzed the effects of a low dose LPS pretreatment on LPS-induced downregulation of PXR mRNA in fetal liver. The results showed that a low dose LPS pretreatment also significantly attenuated LPS-induced downregulation of PXR mRNA in fetal liver (Fig. 7B).

3.6. Effects of maternal LPS exposure on IL-1β and IL-6 in fetal liver

As shown in Table 1, maternal LPS exposure significantly increased the levels of IL-1β (7.65 ± 0.23 pg/mg protein vs. 13.06 ± 0.08 pg/mg protein, $P < 0.01$) and IL-6 (39.48 ± 2.08 pg/mg protein vs. 55.21 ± 1.80 pg/mg protein, $P < 0.01$) in fetal liver.

4. Discussion

CYP3A is a member of the cytochrome P-450 monooxygenase superfamily, which is responsible for the oxidative metabolism of numerous clinically used drugs (Goodwin et al., 2002). The present

Table 1
The effects of maternal LPS exposure on IL-1β and IL-6 in fetal liver

Groups	*n*	IL-1β (pg/mg protein, $\bar{x} \pm$ S.E.M.)	IL-6 (pg/mg protein, $\bar{x} \pm$ S.E.M.)
Saline	12	7.7 ± 0.23	39.5 ± 2.08
LPS	12	13.1 ± 0.08**	55.2 ± 1.80**

** $P < 0.01$ as compared with saline group.

study showed that the level of *cyp3a11* mRNA and CYP3A protein was very low in fetal liver from gd 14 to 16. The amount of CYP3A gradually increased with the advancement of embryonic development. On gd 18, CYP3A protein was about 25% of that in adult liver, whereas *cyp3a11* mRNA in fetal liver was about 55% of that in adult liver. Several studies have demonstrated that LPS downregulated the expression of CYP3A in adult liver (Beigneux et al., 2002; Sachdeva et al., 2003; Xu et al., 2004). The present study found that the expression CYP3A protein in fetal liver was almost completely inhibited when the pregnant mice were administered 0.5 mg/kg of LPS on gd 17. These results are in agreement with our earlier study, in which we showed that perinatal LPS exposure inhibited the expression of *cyp3a11* mRNA and catalytic activity of erythromycin N-demethylation in fetal liver (Xu et al., 2005b).

An in vitro study showed that TNF-α downregulated the expression of CYP3A in cultures of rodent and human hepatocytes (Aitken and Morgan, 2007). However, it remains unsure of the role of TNF-α in LPS-induced downregulation of CYP3A in mouse liver (Aitken et al., 2006). An earlier study using a TNF-α receptor-knockout mouse model showed that TNF-α appeared to mediate LPS-induced downregulation of CYP2D9 and CYP2E1, but not CYP1A, CYP2B, CYP3A, and CYP4A (Warren et al., 1999). On the contrary, a recent study demonstrates that TNF-α plays an important role in LPS-induced downregulation of hepatic *cyp3a11* in IL-6 knockout mice (Ashino et al., 2004). To investigate the role of TNF-α on LPS-induced downregulation of *cyp3a11* in fetal liver, the pregnant mice were pretreated with PTX, an inhibitor of TNF-α synthesis, to inhibit TNF-α production. We found that PTX pretreatment significantly attenuated LPS-induced downregulation of *cyp3a11* mRNA and CYP3A protein in fetal liver. Indeed, the present study showed that maternal LPS exposure increased the levels of TNF-α in maternal serum and amniotic fluid. Interestingly, maternal LPS exposure also significantly increased the levels of TNF-α in fetal liver. A recent study has demonstrated that murine fetal liver expresses high levels of toll-like receptor (TLR)-4 mRNA (Harju et al., 2001). The in vitro studies showed that fetal Kupffer cells secreted TNF-α in response to LPS (Kutteh et al., 1991). However, it remains controversial whether LPS injected into mothers can pass through placenta to fetuses. An earlier study found that an injection with ^{125}I-labeled LPS into the pregnant mice resulted in considerable levels of radioactivity in fetuses (Kohmura et al., 2000). Conversely, a recent study showed that maternally administered LPS could not pass through rat placenta to fetuses (Ashdown et al., 2006). To determine the source of TNF-α in fetal liver, we measured the expression of TNF-α mRNA in maternal liver, placenta and fetal liver. Interestingly, no significant difference in TNF-α mRNA in fetal liver was observed among different groups, whereas maternal LPS exposure significantly upregulated the expression of TNF-α mRNA in maternal liver and placenta. These results indicate that the increased level of TNF-α protein in fetal liver, transferred from either the maternal circulation or amniotic fluid, contributes, at least partially, to LPS-induced downregulation of CYP3A in fetal liver.

A low dose LPS pretreatment has been shown to induce a reduced sensitivity to subsequent challenge of LPS, termed LPS tolerance or LPS hyporesponsiveness (Nomura et al., 2000). LPS tolerance was observed in vivo febrile response and escape from lethality as well as in vitro with a reduced production of inflammatory cytokines in response to a secondary stimulation with LPS (Erroi et al., 1993; Medvedev et al., 2000). Recently, we found that LPS-induced intra-uterine fetal death was alleviated by a low dose LPS pretreatment (Xu et al., 2007). In the present study, we showed that a low dose LPS pretreatment prevented LPS-induced downergulation of cyp3a11 mRNA in fetal liver. Correspondingly, pretreatment with a low dose LPS significantly attenuated LPS-induced downregulation of CYP3A protein in fetal liver. Our earlier study showed that LPS-induced downregulation of *cyp3a11* mRNA in fetal liver was associated with decrease in expression of *pregnane X receptor* (*pxr*) mRNA in fetal liver (Xu et al., 2005b). The present study found that a low dose LPS pretreatment also significantly attenuated LPS-induced downregulation of *pxr* mRNA in fetal liver. To explore why a low dose LPS pretreatment protects fetuses against LPS-induced downregulation of hepatic *cyp3a11* and *pxr* expression, we measured the level of TNF-α in maternal serum and amniotic fluid. We found that a low dose LPS pretreatment attenuated LPS-evoked release of TNF-α in maternal serum and amniotic fluid. Indeed, an earlier in vitro study showed that LPS tolerance in mouse peritoneal macrophages was associated with downregulation of toll-like receptor 4 (Nomura et al., 2000). The present study found that a low dose LPS pretreatment counteracted LPS-induced upregulation of TNF-α mRNA in maternal liver and placenta. Although a low dose LPS pretreatment alleviated LPS-induced increase in TNF-α protein in fetal liver, it had little effect on the expression of TNF-α mRNA in fetal liver. These results suggest that a low dose LPS pretreatment protects fetuses against LPS-induced downregulation of hepatic *cyp3a11* and *pxr* expression through the repression of maternally sourced TNF-α production.

Previous studies have demonstrated that other proinflammatory cytokines, such as IL-1β and IL-6, downregulate the expression of CYP3A in cultures of rodent and human hepatocytes (Abdel-Razzak et al., 1993; Pascussi et al., 2000). The in vivo studies found that these cytokines were also involved in LPS-induced downregulation of CYPs in adult liver (Siewert et al., 2000). The present study showed that maternal LPS exposure significantly increased the levels of IL-1β and IL-6 in fetal liver. Thus, our results do not exclude the involvement of other proinflammatory cytokines. In addition, as noted above, PTX or a low dose LPS pretreatment did attenuate but not completely blocked LPS-induced downregulation of CYP3A in fetal liver suggesting that TNF-α only partially contribute to LPS-induced downregulation of PXR and CYP3A. Indeed, although PTX is generally accepted as a TNF-α antagonist, it is also considered a hydroxyl radical scavenger (Freitas and Filipe, 1995; Franzini et al., 1996). Our earlier study showed that alpha-phenyl-*N*-*t*-butylnitrone (PBN), a free radical spin trapping agent, attenuated LPS-induced downregulation of *pxr* and *cyp3a11* mRNA in fetal liver (Xu et al., 2005b). Therefore, the present study does not exclude the involvement of other mechanism, such as oxidative stress, in LPS-induced downregulation of CYP3A in fetal liver.

In summary, the present results allow us to reach the following conclusions. First, maternal LPS exposure downregulates the expression of CYP3A in fetal liver; second, TNF-α, possibly sourced from maternal circulation and perhaps from amniotic fluid, contributes, at least in part, to LPS-induced downregulation of CYP3A in fetal liver, and third, a low dose LPS pretreatment attenuates LPS-induced downregulation of CYP3A in fetal liver through inhibiting the release of maternally sourced TNF-α. These results provide new evidence that a low dose LPS pretreatment protects fetuses against high-dose LPS-induced developmental toxicity.

Acknowledgements

This project was supported by National Natural Science Foundation of China (30371667, 30572223, 30671786) and Anhui Provincial Natural Science Foundation (050430714).

References

Abdel-Razzak, Z., Loyer, P., Fautrel, A., Gautier, J.C., Corcos, L., Turlin, B., Beaune, P., Guillouzo, A., 1993. Cytokines down-regulate expression of major cytochrome P-450 enzymes in adult human hepatocytes in primary culture. Mol. Pharmacol. 44, 707–715.

Aitken, A.E., Richardson, T.A., Morgan, E.T., 2006. Regulation of drug-metabolizing enzymes and transporters in inflammation. Annu. Rev. Pharmacol. Toxicol. 46, 123–149.

Aitken, A.E., Morgan, E.T., 2007. Gene-specific effects of inflammatory cytokines on cytochrome P4502C, 2B6 and 3A4 mRNA levels in human hepatocytes. Drug Metab. Dispos. 35, 1687–1693.

Anakk, S., Kalsotra, A., Shen, Q., Vu, M.T., Staudinger, J.L., Davies, P.J., Strobel, H.W., 2003. Genomic characterization and regulation of CYP3a13: role of xenobiotics and nuclear receptors. FASEB J. 17, 1736–1738.

Ashdown, H., Dumont, Y., Ng, M., Poole, S., Boksa, P., Luheshi, G.N., 2006. The role of cytokines in mediating effects of prenatal infection on the fetus: implications for schizophrenia. Mol. Psychiatry 11, 47–55.

Ashino, T., Oguro, T., Shioda, S., Horai, R., Asano, M., Sekikawa, K., Iwakura, Y., Numazawa, S., Yoshida, T., 2004. Involvement of interleukin-6 and tumor necrosis factor alpha in CYP3A11 and 2C29 down-regulation by Bacillus Calmette-Guerin and lipopolysaccharide in mouse liver. Drug Metab. Dispos. 32, 707–714.

Beigneux, A.P., Moser, A.H., Shigenaga, J.K., Grunfeld, C., Feingold, K.R., 2002. Reduction in cytochrome P-450 enzyme expression is associated with repression of CAR (constitutive androstane receptor) and PXR (pregnane X receptor) in mouse liver during the acute phase response. Biochem. Biophys. Res. Commun. 293, 145–149.

Chen, Y.H., Wang, J.P., Wang, H., Sun, M.F., Wei, L.Z., Wei, W., Xu, D.X., 2005. Lipopolysaccharide treatment downregulates the expression of the pregnane X receptor, cyp3a11 and mdr1a genes in mouse placenta. Toxicology 211, 242–252.

Chen, Y.H., Xu, D.X., Wang, J.P., Wang, H., Wei, L.Z., Sun, M.F., Wei, W., 2006. Melatonin protects against lipopolysaccharide-induced intra-uterine fetal death and growth retardation in mice. J. Pineal Res. 40, 40–47.

Choudhary, D., Jansson, I., Schenkman, J.B., Sarfarazi, M., Stoilov, I., 2003. Comparative expression profiling of 40 mouse cytochrome P450 genes in embryonic and adult tissues. Arch. Biochem. Biophys. 414, 91–100.

de Wildt, S.N., Kearns, G.L., Leeder, J.S., van den Anker, J.N., 1999. Cytochrome P450 3A: ontogeny and drug disposition. Clin. Pharmacokinet. 37, 485–505.

Dzierzak, E., Medvinsky, A., 1995. Mouse embryonic hematopoiesis. Trends Genet. 11, 359–366.

Erroi, A., Fantuzzi, G., Mengozzi, M., Sironi, M., Orencole, S.F., Clark, B.D., Dinarello, C.A., Isetta, A., Gnocchi, P., Giovarelli, M., 1993. Differential regulation of cytokine production in lipopolysaccharide tolerance in mice. Infect. Immun. 61, 4356–4359.

Franzini, E., Sellak, H., Marquetty, C., Babin-Chevaye, C., Hakim, J., Pasquier, C., 1996. Inhibition of human neutrophil binding to hydrogen peroxide-treated endothelial cells by cAMP and hydroxyl radical scavengers. Free Radic. Biol. Med. 21, 15–23.

Freitas, J.P., Filipe, P.M., 1995. Pentoxifylline. A hydroxyl radical scavenger. Biol. Trace Elem. Res. 47, 307–311.

Goodwin, B., Redinbo, M.R., Kliewer, S.A., 2002. Regulation of cyp3a gene transcription by the pregnane x receptor. Annu. Rev. Pharmacol. Toxicol. 42, 1–23.

Hardy, R.R., Hayakawa, K., 2001. B cell development pathways. Annu. Rev. Immunol. 19, 595–621.

Harju, K., Glumoff, V., Hallman, M., 2001. Ontogeny of Toll-like receptors Tlr2 and Tlr4 in mice. Pediatr. Res. 49, 81–83.

Hines, R.N., McCarver, D.G., 2002. The ontogeny of human drug-metabolizing enzymes: phase I oxidative enzymes. J. Pharmacol. Exp. Ther. 300, 355–360.

Hulla, J.E., Juchau, M.R., 1989. Developmental aspects of P450IIIA: prenatal activity and inducibility. Drug Metab. Rev. 20, 765–779.

Kohmura, Y., Kirikae, T., Kirikae, F., Nakano, M., Sato, I., 2000. Lipopolysaccharide (LPS)-induced intra-uterine fetal death (IUFD) in mice is principally due to maternal cause but not fetal sensitivity to LPS. Microbiol. Immunol. 44, 897–904.

Krauer, B., Dayer, P., 1991. Fetal drug metabolism and its possible clinical implications. Clin. Pharmacokinet. 21, 70–80.

Kutteh, W.H., Rainey, W.E., Carr, B.R., 1991. Glucocorticoids inhibit lipopolysaccharide-induced production of tumor necrosis factor-alpha by human fetal Kupffer cells. J. Clin. Endocrinol. Metab. 73, 296–301.

Medvedev, A.E., Kopydlowski, K.M., Vogel, S.N., 2000. Inhibition of lipopolysaccharide-induced signal transduction in endotoxin-tolerized mouse macrophages: dysregulation of cytokine, chemokine, and toll-like receptor 2 and 4 gene expression. J. Immunol. 164, 5564–5574.

Morgan, E.T., Li-Masters, T., Cheng, P.Y., 2002. Mechanisms of cytochrome P450 regulation by inflammatory mediators. Toxicology 181–182, 207–210.

Nomura, F., Akashi, S., Sakao, Y., Sato, S., Kawai, T., Matsumoto, M., Nakanishi, K., Kimoto, M., Miyake, K., Takeda, K., Akira, S., 2000. Cutting edge: endotoxin tolerance in mouse peritoneal macrophages correlates with down-regulation of surface toll-like receptor 4 expression. J. Immunol. 164, 3476–3479.

Pascussi, J.M., Gerbal-Chaloin, S., Pichard-Garcia, L., Daujat, M., Fabre, J.M., Maurel, P., Vilarem, M.J., 2000. Interleukin-6 negatively regulates the expression of pregnane X receptor and constitutively activated receptor in primary human hepatocytes. Biochem. Biophys. Res. Commun. 274, 707–713.

Rich, K.J., Boobis, A.R., 1997. Expression and inducibility of P450 enzymes during liver ontogeny. Microsc. Res. Tech. 39, 424–435.

Sachdeva, K., Yan, B., Chichester, C.O., 2003. Lipopolysaccharide and cecal ligation/puncture differentially affect the subcellular distribution of the pregnane X receptor but consistently cause suppression of its target genes CYP3A. Shock 19, 469–474.

Siewert, E., Bort, R., Kluge, R., Heinrich, P.C., Castell, J., Jover, R., 2000. Hepatic cytochrome P450 down-regulation during aseptic inflammation in the mouse is interleukin 6 dependent. Hepatology 32, 49–55.

Stevens, J.C., Hines, R.N., Gu, C., Koukouritaki, S.B., Manro, J.R., Tandler, P.J., Zaya, M.J., 2003. Developmental expression of the major human hepatic CYP3A enzymes. J. Pharmacol. Exp. Ther. 307, 573–582.

Warren, G.W., Poloyac, S.M., Gary, D.S., Mattson, M.P., Blouin, R.A., 1999. Hepatic cytochrome P-450 expression in tumor necrosis factor-α receptor (p55/p75) knockout mice after endotoxin administration. J. Pharmacol. Exp. Ther. 288, 945–950.

Xu, D.X., Wei, W., Sun, M.F., Wu, C.Y., Wang, J.P., Wei, L.Z., Zhou, C.F., 2004. Kupffer cells and ROS partially mediate LPS-induced down-regulation of nuclear receptor Pregnane X receptor and its target gene CYP3A in mouse liver. Free Radic. Biol. Med. 37, 10–22.

Xu, D.X., Chen, Y.H., Wang, H., Zhao, L., Wang, J.P., Wei, W., 2005a. Effect of N-acetylcysteine on lipopolysaccharide-induced intra-uterine fetal death and intra-uterine growth retardation in mice. Toxicol. Sci. 88, 525–533.

Xu, D.X., Chen, Y.H., Wang, J.P., Sun, M.F., Wang, H., Wei, L.Z., Wei, W., 2005b. Perinatal lipopolysaccharide exposure downregulates pregnane X receptor and cyp3a11 expression in fetal mouse liver. Toxicol. Sci. 87, 38–45.

Xu, D.X., Chen, Y.H., Zhao, L., Wang, H., Wei, W., 2006. Reactive oxygen species are involved in lipopolysaccharide-induced intra-uterine growth restriction and skeletal development retardation in mice. Am. J. Obstet. Gynecol. 195, 1707–1714.

Xu, D.X., Wang, H., Zhao, L., Ning, H., Zhang, C., Chen, Y.H., 2007. Effects of low-dose lipopolysaccharide (LPS) pretreatment on LPS-induced intra-uterine fetal death and preterm labor. Toxicology 234, 167–175.

Zhou, Z., Wang, L., Song, Z., Lambert, J.C., McClain, C.J., Kang, Y.J., 2003. A critical involvement of oxidative stress in acute alcohol-induced hepatic TNF-alpha production. Am. J. Pathol. 163, 1137–1146.