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

Yuan-Hua Chen [a], Jian-Ping Wang [a], Hua Wang [a], Mei-Fang Sun [a], Ling-Zhen Wei [a], Wei Wei [b,c], De-Xiang Xu [a,b,c,*]

[a] *Department of toxicology, Anhui Medical University, Hefei 230032, PR China*
[b] *Institute of Clinical Pharmacology, Anhui Medical University, Hefei 230032, PR China*
[c] *Key Laboratory of Anti-inflammatory and Immunopharmacology of Anhui Province, Hefei 230032, PR China*

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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

* Corresponding author. Tel.: +86 551 5161170.
E-mail address: xudex@mail.hf.ah.cn (D.-X. Xu).

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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.

2. Materials and methods

2.1. Chemicals

Lipopolysaccharide (*Escherichia coli* LPS, serotype 0127:B8), alpha-phenyl-*N*-*t*-butylnitrone (PBN) and *N*-acetylcysteine (NAC) 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 whose foundation colonies were all introduced from Charles River Laboratories Inc. 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) 0.5. The present study included three separate experiments.

2.2.1. Experiment 1

To investigate the effects of LPS on *PXR*, *cyp3a* and *mdr1a* gene expressions in mouse placenta, gestational day 17 ICR mice were injected with different doses of LPS (0.1, 0.2 and 0.5 mg/kg, i.p.). The saline-treated pregnant mice served as controls. The mice were sacrificed at 12 h after LPS treatment. Mouse placenta was excised for total RNA extraction.

2.2.2. Experiment 2

To investigate the effects of PBN on LPS-induced downregulation of *PXR*, *cyp3a* and *mdr1a* gene expressions in mouse placenta, pregnant mice were divided into four groups randomly. The pregnant mice in LPS-treated group were injected with 0.2 mg/kg of LPS (i.p.) on gd 17. The pregnant mice in LPS + PBN group were injected with 100 mg/kg of PBN (i.p.) 30 min before and 3 h after LPS administration (0.2 mg/kg, i.p.). A saline-treated and PBN-treated pregnant mice served as controls. Three pregnant mice (one-third of the total) were sacrificed for the measurements of TNF-α, IL-1β and IL-6 mRNAs at 2 h after LPS treatment. Three pregnant mice (one-third of the total) were sacrificed at 6 h after LPS treatment. Mouse placentas were excised for the measurements of thiobarbituric acid-reactive substance (TBARS) contents. Three pregnant mice (one-third of the total) were sacrificed at 12 h after LPS treatment. Mouse placentas were excised for the measurements of *PXR*, *cyp3a11* and *mdr1a* mRNA.

2.2.3. Experiment 3

To investigate the effects of NAC on LPS-induced downregulation of *PXR*, *cyp3a* and *mdr1a* gene expression in mouse placenta, pregnant mice were divided into four groups randomly. The pregnant mice in LPS group were injected with 0.2 mg/kg of LPS (i.p.) on gd 17. Pregnant mice in LPS + NAC group were injected with 100 mg/kg of NAC (i.p.) at 30 min before and 3 h after LPS (0.2 mg/kg, i.p.) treatment. Saline- or NAC-treated pregnant mice served as controls. Three pregnant mice (one-third of the total) were sacrificed for the measurements of placental TNF-α, IL-1β and IL-6 mRNAs at 2 h after LPS treatment. Three pregnant mice (one-third of the total) were sacrificed at 6 h after LPS treatment. Mouse placentas were excised for the measurements of thiobarbituric acid-reactive substance and GSH contents. Three pregnant mice (one-third of the total) were sacrificed at 12 h after LPS treatment. Mouse placentas were excised for the measurements of *PXR*, *cyp3a11* and *mdr1a* mRNA.

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. Isolation of total RNA and RT

About 50 mg of placental tissue was collected from each sample. Total cellular RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RNase-free DNase (Promega) was used to remove genomic DNA. The integrity and concentration of RNA was determined by measuring absorbance at 260 nm followed by electrophoresis on agarose gels. Total RNA was stored at −80 °C. For the synthesis of cDNA, total RNA (2.0 μg) from each sample was resuspended in a 20-μl final volume of reaction buffer, which contained 25 mM Tris–HCl, pH 8.3, 37.5 mM KCl,

Table 1
Oligonucleotide primers used in PCR

Name	Dir	Oligonucleotide sequence (5′–3′)	Size (bp)	Reference
gapdh	F	GAG GGG CCA TCC ACA GTC TTC	340	Xu et al. (2004)
	R	CAT CAC CAT CTT CCA GGA GCG		
PXR	F	GCG CGG AGA AGA CGG CAG CAT C	254	Li et al. (2000)
	R	CCC AGG TTC CCG TTT CCG TGT C		
cyp3a11	F	CTC AAT GGT GTG TAT ATC CCC	423	Ueda et al. (2002)
	R	CCG ATG TTC TTA GAC ACT GCC		
mdr1a	F	AGC ATC TGT GAA CCA CAT	249	Veau et al. (2002)
	R	GTT GCT GTT CTA CCG CTG G		
TNF-α	F	GGC AGG TCT ACT TTG GAG TCA TTG C	307	Murray et al. (1990)
	R	ACA TTC GAG GCT CCA GTG AAT TCG G		
IL-1β	F	TCA TGG GAT GAT GAT GAT AAC CTG CT	502	Ehlers et al. (1992)
	R	CCC ATA CTT TAG GAA GAC ACG GAT T		
IL-6	F	CTG GTG ACA ACC ACG GCC TTC CCT A	600	Ehlers et al. (1992)
	R	ATG CTT AGG CAT AAC GCA CTA GGT T		

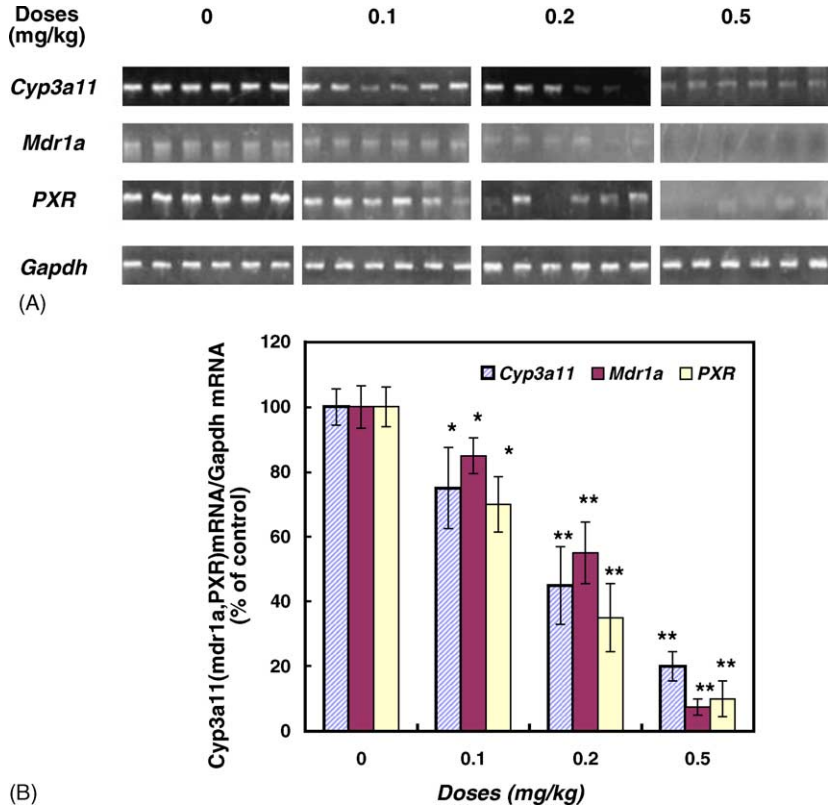

Fig. 1. The effects of LPS on *PXR*, *Cyp3a11* and *Mdr1a* mRNA expressions in placenta. (A) Pregnant mice were injected with different doses of LPS (0.1–0.5 mg/kg, i.p.) on gd 17. Placentas were excised and total RNA was extracted at 12 after LPS treatment. *PXR*, *Cyp3a11* and *Mdr1a* mRNA levels were determined using RT-PCR. Six samples each group were representatives of 12 placentas from three dams. (B) Quantitative analysis of *PXR*, *Cyp3a11* and *Mdr1a* mRNA levels on 12 placentas from three dams at each point was performed. The *PXR*, *Cyp3a11* and *Mdr1a* mRNA levels were normalized to *Gapdh* mRNA level in the same samples. The *PXR*, *Cyp3a11* and *Mdr1a* mRNA levels of the control was set at 100%. $^{*}P < 0.05$ and $^{**}P < 0.01$ as compared with control group.

10 mM dithiothreitol, 1.5 mM $MgCl_2$, 10 mM of each dNTP and 0.5 mg oligo$(dT)_{15}$ primer (Promega). After the reaction mixture reached 38 °C, 400 units of RT (Promega) was added to each tube and the sample was incubated for 60 min at 38 °C. Reverse transcription was stopped by denaturing the enzyme at 95 °C.

2.4. PCR amplification

The final PCR mixture contained 2.5 μl of cDNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTP mixture, 1 U of Taq DNA polymerase, 1 μM sense and antisense primers and sterile water to 50 μl. The reaction mixture was covered with mineral oil. PCR for *glyceraldehyde-3-phosphate dehydrogenase* (*gapdh*) was performed on each individual sample as an internal positive-control standard. Following primers were synthesized by Shanghai Sangon Biological Engineering Technology and Service Company (Shanghai, China), according to sequence designs previously described (Table 1). Number of cycles and annealing temperature were optimized for each primer pair. For *gapdh* and *cyp3a11*, amplification was initiated by 3 min of denaturation at 94 °C for 1 cycle, followed by 30 cycles at 94 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min. For *mdr1a*, amplification was initiated by 3 min of denaturation at 94 °C for 1 cycle, followed by 30 cycles at 94 °C for 30 s, 53 °C for 30 s and 72 °C for 1 min. For *PXR*, amplification was initiated by 3 min of denaturation at 94 °C for 1 cycle, followed by 45 cycles at 94 °C for 30 s, 68 °C for 30 s and 72 °C for 1 min. For TNF-α and IL-1β, amplification was initiated by 3 min of denaturation at 94 °C for 1 cycle, followed by 30 cycles each of denaturation at 94 °C for 45 s, annealing of primer and fragment at 60 °C for 45 s and primer extension at 72 °C for 1 min. For IL-6, amplification was initiated by 3 min of denaturation at 94 °C for 1 cycle, followed by 35 cycles each of denaturation at 94 °C for 45 s, annealing of primer and fragment at 60 °C for 45 s and primer extension at 72 °C for 1 min. A final extension of 72 °C for 10 min was included. The amplified PCR products were subjected to electrophoresis at 75 V through 1.5% agarose gels (Sigma) for 45 min. The pBR322 DNA digested with Alu I was used for molecular markers (MBI Fermentas). Agarose gels were stained with 0.5 mg/ml ethidium bromide (Sigma) TBE buffer.

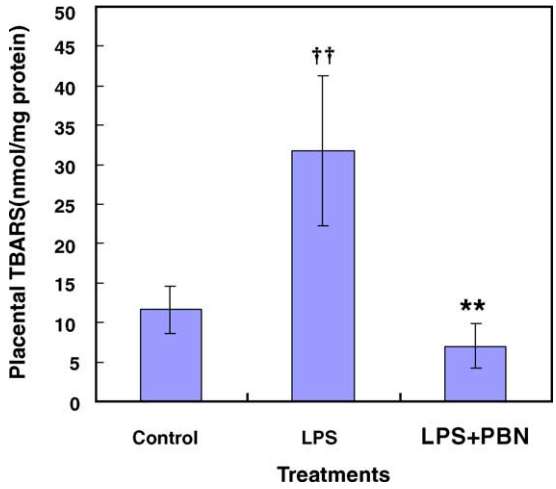

Fig. 2. The effects of PBN on LPS-induced *lipid peroxidation* in mouse placenta. Pregnant mice were injected with PBN (100 mg/kg, i.p.) at 30 min before and 3 h after LPS (0.2 mg/kg, i.p.) treatment. Placenta were excised and homogenized at 6 h after LPS treatment. TBARS content was measured as described in Section 2. Data were expressed as means ± S.E.M. ($n = 9$). $^{\dagger\dagger}P < 0.01$ as compared with control group. $^{**}P < 0.01$ as compared with LPS-treated group.

2.5. Determination of glutathione content

The glutathione (GSH) was determined by the method of Griffith (1980). Proteins of 0.4 ml tissue homogenates were precipitated by the addition of 0.4 ml of a metaphosphoric acid solution. After 40 min, the protein precipitate was separated from the remaining solution by centrifugation at 5000 rpm at 4 °C for 5 min. Four hundred microliters of the supernatant was combined with 0.4 ml of 300 mM Na_2HPO_4, and the absorbance at 412 nm was read against a blank consisting of 0.4 ml supernatant plus 0.4 ml H_2O. Then, 100 μl DTNB (0.02%, w/v; 20 mg DTNB in 100 ml of 1% sodium citrate) was added to the blank and sample and absorbance of the sample was read against the blank at 412 nm. The GSH content was determined using a calibration curve prepared with an authentic sample. GSH values were expressed as nmol mg^{-1} protein. Protein content was measured according to the method of Lowry et al. (1951).

2.6. Lipid peroxidation assay

Lipid peroxidation was quantified by measuring thiobarbituric acid-reactive substance as described

previously (Ohkawa et al., 1979). Tissue was homogenized in nine volumes of 50 mmol/l Tris–HCl buffer (pH 7.4) containing 180 mmol/l KCl, 10 mmol/l EDTA and 0.02% butylated hydroxytoluene. To 0.2 ml of the tissue homogenate, 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid, 1.5 ml of 0.9% thiobarbituric acid and 0.6 ml of distilled water were added and vortexed. The reaction mixture was placed in a water bath at 95 °C for 1 h. After cooling on ice, 1.0 ml of distilled water and 5.0 ml of butanol/pyridine mixture (15:1, v/v) were added and vortexed. After centrifugation at 10,000 × *g* for 10 min, absorbance of the resulting lower phase was determined at 532 nm. The TBARS concentration was calculated using 1,1,5,5-tetraethoxypropane as standard.

2.7. Statistical analysis

The *PXR*, *Cyp3a11* and *Mdr1a* mRNA levels were normalized to *Gapdh* mRNA level in the same samples. The *PXR*, *Cyp3a11* and *Mdr1a* mRNA levels of the control was assigned as 100%. Quantified data from analysis of RT-PCR, GSH and TBARS contents were expressed as means ± S.E.M. at each point. ANOVA

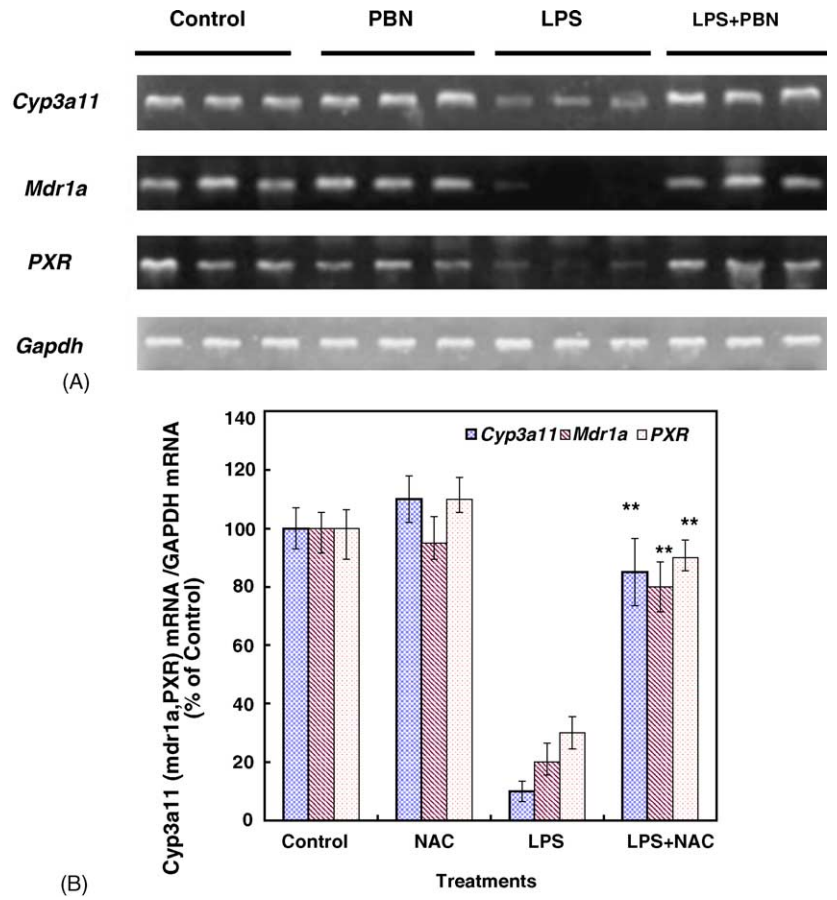

Fig. 3. The effects of PBN on LPS-induced downregulation of *PXR*, *Cyp3a11* and *Mdr1a* mRNA levels in mouse placenta. Pregnant mice were injected with PBN (100 mg/kg, i.p.) at 30 min before and 3 h after LPS (0.2 mg/kg, i.p.) treatment. (A) Placentas were excised and total RNA was extracted at 12 h after LPS treatment. *PXR*, *Cyp3a11* and *Mdr1a* mRNA levels were determined using RT-PCR. Three samples each group were representatives of 12 placentas from three dams. (B) Quantitative analysis of *PXR*, *Cyp3a11* and *Mdr1a* mRNA levels on 12 placentas from three dams at each point was performed. The *PXR*, *Cyp3a11* and *Mdr1a* mRNA levels were normalized to *Gapdh* mRNA level in the same samples. The *PXR*, *Cyp3a11* and *Mdr1a* mRNA levels of control was set at 100%. $^{**}P < 0.01$ as compared with LPS-treated group.

and the Student–Newmann–Keuls post hoc test were used to determine differences between the treated animals and the control and statistical significance.

3. Results and discussion

Cytochrome P450 3A is a member of the cytochrome P450 monooxygenase superfamily, which is responsible for the oxidative metabolism of numerous clinically used drugs. LPS-induced downregulation of CYP3A in liver has been demonstrated in a series of reports (Morgan et al., 2002). Several studies found that CYP3A is detectable in human and mouse placenta as early as the first trimester of pregnancy, which play a key role in the detoxification of drug or other xenobiotics (Hakkola et al., 1996a,b). Therefore, we investigated the effects of LPS on placental *cyp3a11* mRNA levels. Pregnant ICR mice were administered with different doses (0.1–0.5 mg/kg) of LPS on gestational day 17. *Cyp3a11* mRNA levels were determined using RT-PCR at 12 h after LPS treatment. As shown in Fig. 1A and B, LPS significantly decreased placental *cyp3a11* mRNA levels in a dose-dependent manner.

MDR1 gene belongs to the ATP-binding cassette family. Several studies showed that LPS dramatically downregulated the expression of *Mdr1a* in liver, brain and intestine (Hartmann et al., 2001; Goralski et al., 2003; Kalitsky-Szirtes et al., 2004; Cherrington et al., 2004). MDR1 is expressed constantly in placenta, which is a drug efflux transporter that limits the entry of various potentially toxic drugs and xenobiotics into the fetus (Fromm, 2004; Novotna et al., 2004; Leazer and Klaassen, 2003). Present study investigated the effects of LPS on placental *mdr1a* mRNA levels. Our results found that LPS significantly decreased *mdr1a* mRNA levels in a dose-dependent manner (Fig. 1A and B).

Pregnane X receptor is a nuclear receptor that 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). Previous study showed that LPS repressed hepatic PXR mRNA and protein levels. Moreover, LPS-induced downregulation of CYP3A is associated with a marked reduction in the expression of *PXR* in liver (Beigneux et al., 2002; Sachdeva et al., 2003). Recent study found that *PXR* is expressed constitutively in mouse placenta (Teng and Piquette-

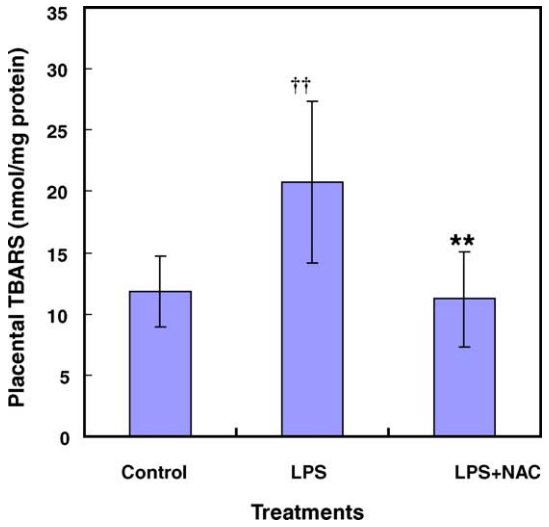

Fig. 4. The effects of NAC on LPS-induced *lipid peroxidation* in mouse placenta. Pregnant mice were injected with NAC at 30 min before and 3 h after LPS (0.2 mg/kg, i.p.) treatment. Placentas were excised and homogenized at 6 h after LPS treatment. TBARS content was measured as described in Section 2. Data were expressed as means ± S.E.M. ($n = 12$). $^{\dagger\dagger}P < 0.01$ as compared with control group. $^{**}P < 0.01$ as compared with LPS-treated group.

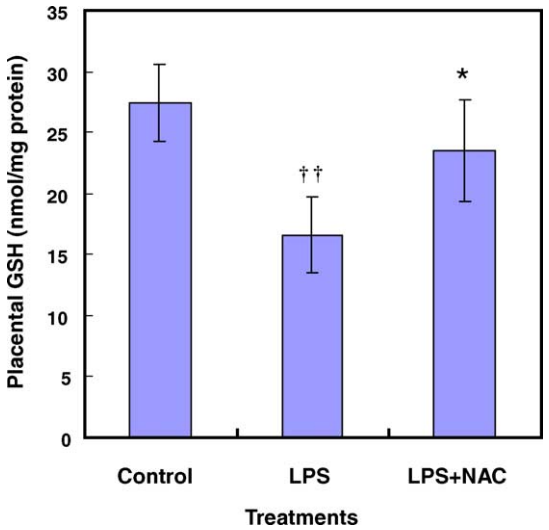

Fig. 5. The effects of NAC on GSH content in placenta. Pregnant mice were injected with NAC (100 mg/kg, i.p.) at 30 min before and 3 h after LPS (0.2 mg/kg, i.p.) treatment. Placentas were excised and homogenized at 6 h after LPS treatment. GSH content was measured as described in Section 2. Data were expressed as means ± S.E.M. ($n = 12$). $^{\dagger\dagger}P < 0.01$ as compared with control group. $^{*}P < 0.05$ as compared with LPS-treated group.

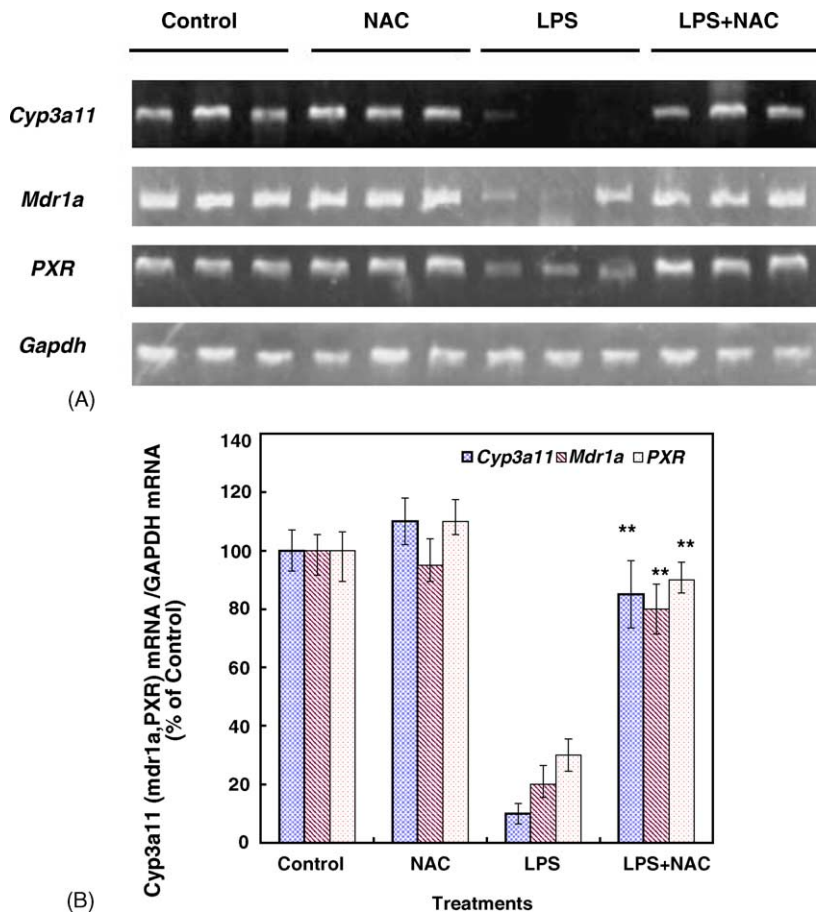

Fig. 6. The effects of NAC on LPS-induced downregulation of *PXR*, *Cyp3a11* and *Mdr1a* mRNA levels in placenta. Pregnant mice were injected with NAC (100 mg/kg, i.p.) at 30 min before and 3 h after LPS (0.2 mg/kg, i.p.) treatment. (A) Placentas were excised and total RNA was extracted at 12 h after LPS treatment. *PXR*, *Cyp3a11* and *Mdr1a* mRNA levels were determined using RT-PCR. Three samples each group were representatives of 12 placentas from three dams. (B) Quantitative analysis of *PXR*, *Cyp3a11* and *Mdr1a* mRNA levels on 12 placentas from three dams at each point was performed. The *PXR*, *Cyp3a11* and *Mdr1a* mRNA levels were normalized to *Gapdh* mRNA level in the same samples. The *PXR*, *Cyp3a11* and *Mdr1a* mRNA level of control was set at 100%. $^{**}P < 0.01$ as compared with LPS-treated group.

Miller, 2005). In this study, we investigated the effects of LPS on placental *PXR* mRNA levels. Consistent with downregulation of cyp3a11 and mdr1a, LPS treatment markedly inhibited the expression of PXR in mouse placenta (Fig. 1A and B).

Reactive oxygen species are known to influence the expressions of a number of genes and signal transduction pathways (Allen and Tresini, 2000). Previous studies found that ROS are involved in LPS-induced downregulation of *PXR* and *cyp3a* in adult mouse liver (Xu et al., 2004, 2005). Several studies demonstrated that LPS significantly increased placental 4-hydroxy-2-nonenal (HNE)-modified proteins, an indicator of oxidative stress and nitrotyrosine, a marker for $O_2^{\bullet -}$, NO and $ONOO^-$ formation (Miller et al., 1996; Ejima et al., 1999; Asagiri et al., 2003). In this study, we investigated LPS-induced lipid peroxidation in mouse placenta. Results showed that LPS significantly augmented thiobarbituric acid-reactive substance levels in mouse placenta (Fig. 2). To determine the role of ROS on LPS-induced downregulation of placental *PXR*, *cyp3a11* and *mdr1a* gene expressions, pregnant mice were treated with alpha-phenyl-*N*-*t*-butylnitrone before and after LPS administration. As expected, PBN, as a

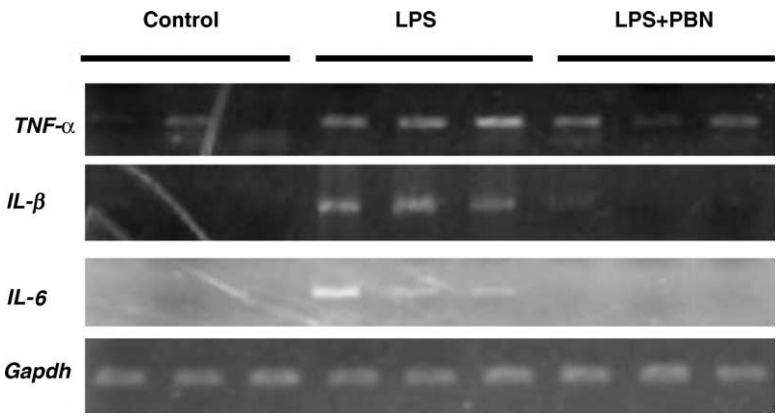

Fig. 7. The effects of PBN on LPS-induced *proinflammatory cytokine expressions* in mouse placenta. Pregnant mice were injected with PBN (100 mg/kg, i.p.) at 30 min before LPS (0.2 mg/kg, i.p.) treatment. Placentas were excised and total RNA was extracted at 2 h after LPS treatment. TNF-α, IL-1β and IL-6 mRNA levels in placentas were determined using RT-PCR. Three samples each group were representatives of 12 placentas from three dams.

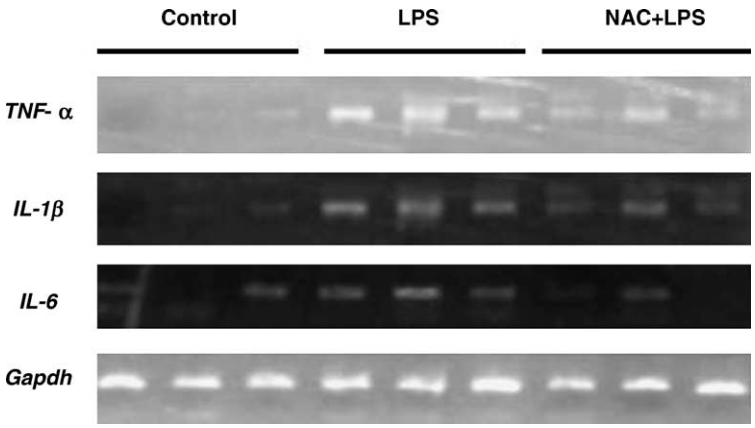

Fig. 8. The effects of NAC on LPS-induced *proinflammatory cytokine expressions* in mouse placenta. Pregnant mice were injected with NAC (100 mg/kg, i.p.) at 30 min before LPS (0.2 mg/kg, i.p.) treatment. Placentas were excised and total RNA was extracted at 2 h after LPS treatment. TNF-α, IL-1β and IL-6 mRNA levels in placentas were determined using RT-PCR. Three samples each group were representatives of 12 placentas from three dams.

free radical spin trapping agent, significantly attenuated LPS-induced lipid peroxidation in mouse placenta (Fig. 2). In accordance with its antioxidative effects, PBN significantly attenuated LPS-induced downregulation of *PXR*, *cyp3a11* and *mdr1a* mRNA in mouse placenta (Fig. 3A and B). These results suggest that ROS may mediate LPS-induced downregulation of *PXR*, *cyp3a11* and *mdr1a* gene expressions in mouse placenta.

N-acetylcysteine is a glutathione precursor and a direct antioxidant. To investigate the protective effects of NAC on LPS-induced downregulation of placental *PXR*, *cyp3a11* and *mdr1a* mRNA expressions, pregnant mice were treated with NAC before and after LPS administration. The effects of NAC on LPS-induced TBARS and GSH contents in mouse placenta were measured at 6 h after LPS treatment. Results showed that NAC attenuated LPS-induced TBARS (Fig. 4) and GSH depletion (Fig. 5) in mouse placenta. Furthermore, the effects of NAC on LPS-induced *PXR*, *cyp3a11* and *mdr1a* gene downregulation were determined at 12 h after LPS treatment. Results showed that

NAC significantly attenuated LPS-induced downregulation of *PXR*, *cyp3a11* and *mdr1a* mRNA levels in mouse placenta (Fig. 6).

Numerous studies demonstrated that proinflammatory cytokines, such as TNF-α, IL-1β and IL-6 were involved in LPS-induced downregulation of cytochrome P450s (Sewer et al., 1997; Monshouwer et al., 1996). The present study showed that LPS induced TNF-α, IL-1β and IL-6 mRNA expressions in mouse placenta (Figs. 7 and 8). These results are in agreement with the earlier work by others (Bell et al., 2004; Gayle et al., 2004), in which placental TNF-α, IL-1β and IL-6 mRNA levels significantly increased after pregnant rats were treated with LPS. Furthermore, the present study also found that PBN and NAC treatments significantly attenuated LPS-induced TNF-α, IL-1β and IL-6 expressions in mouse placenta. Therefore, our results do not exclude the involvement of proinflammatory cytokines in LPS-induced downregulation of *PXR*, *cyp3a11* and *mdr1a* gene expressions in mouse placenta.

In summary, the present results allow us to reach the following conclusions. First, LPS downregulates *pregnane X receptor*, *cyp3a11* and *mdr1a* expressions in mouse placenta; second, reactive oxygen species may be involved in LPS-induced downregulation of placental *PXR*, *cyp3a11* and *mdr1a* gene expressions; and third, NAC prevents LPS-induced downregulation of *PXR*, *cyp3a11* and *mdr1a* in mouse placenta via counteracting LPS-induced oxidative stress.

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