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Lipopolysaccharide downregulates the expressions of intestinal *pregnane X receptor* and *cytochrome P450 3a11*

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

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

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Abstract

The pregnane X receptor is a member of the nuclear receptor superfamily, which heterodimerize with the retinoid X receptor, and is an important regulator of cytochrome P450 3A (CYP3A). Lipopolysaccharide (LPS)-induced downregulation of *pregnane X receptor* and its target gene *cyp3a11* has been well characterized in mouse liver. In the present study, we investigated the effects of LPS on the expressions of *pregnane X receptor* and its target gene *cyp3a11* in mouse intestine. Mice were injected intraperitoneally with different doses of LPS (0.1–5.0 mg/kg). Intestinal *pregnane X receptor*, *retinoid X receptor αlpha* and *cyp3a11* mRNA were determined using reverse transcription polymerase chain reaction (RT-PCR). Erythromycin *N*-demethylase (ERND) activity was used as an indicator of CYP3A expression. Results showed that LPS significantly downregulated the expressions of intestinal *pregnane X receptor* and its heterodimer *retinoid X receptor alpha* in a dose-dependent manner. Furthermore, LPS repressed the upregulation of *cyp3a11* mRNA and ERND catalytic activity in mice pretreated with pregnane X receptor ligand dexamethasone. Additional experiment showed that LPS significantly increased the level of intestinal thiobarbituric acid-reactive substance, which was attenuated by oral administration with either *N*-acetylcysteine or ascorbic acid. Correspondingly, oral administration with either *N*-acetylcysteine or ascorbic acid significantly attenuated LPS-induced downregulation of intestinal *pregnane X receptor* and *retinoid X receptor αlpha*. In addition, these antioxidants prevented the repressive effect of LPS on dexamethasone-inducible *cyp3a11* mRNA and ERND activity in mouse intestine. Taken together, these results indicate that LPS suppresses the expressions of *pregnane X receptor* and its target gene *cyp3a11* in mouse intestine. LPS-induced downregulation of *pregnane X receptor* and *cyp3a11* in mouse intestine is mediated, at least in part, by oxidative stress.

Keywords: Lipopolysaccharide (LPS); Pregnane X receptor; Retinoid X receptor; Cytochrome P450 3A (CYP3A); Erythromycin *N*-demethylase (ERND); Intestine

1. Introduction

The cytochrome P450 3A (CYP3A) is a member of the cytochrome P450 monooxygenase superfamily, which is responsible for the oxidative metabolism of numerous clinically used drugs (Thummel and Wilkinson, 1998). CYP3A enzymes are expressed at high levels in hepatocytes and mature villus tip enterocytes (Kolars et al., 1994). It has been understood that CYP3A is inducible by an array of structurally diverse compounds, including naturally occurring and synthetic glucocorticoids, pregnane compounds, and macrolide antibiotics (Kliewer et al., 2002). Recent research showed that *CYP3A* gene expression is highly regulated by development, tissue-specific factors, hormonal influences and pathophysiological mechanisms (Quattrochi and Guzelian, 2001; Goodwin et al., 2002).

Pregnane X receptor is a member of the nuclear receptor family of ligand-activated transcription factors that include the steroid, retinoid and thyroid hormone receptors as well as many orphan receptors for which physiological ligands have yet to be identified (Mangelsdorf et al., 1995; Giguere, 1999). *Pregnane X receptor* is highly expressed in the liver and intestine, where *CYP3A* genes are most highly expressed and induced (Zhang et

* Corresponding author. Department of toxicology, Anhui Medical University, Hefei, 230032, PR China. Tel.: +86 551 5161170; fax: +86 551 5161179.
E-mail address: xudex@mail.hf.ah.cn (D.-X. Xu).

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al., 1999; Kliewer et al., 1998). Furthermore, numerous studies demonstrated that pregnane X receptor, which heterodimerize with the retinoid X receptor, is an important regulator of CYP3A (Lehmann et al., 1998; Bertilsson et al., 1998). CYP3A inducers, such as dexamethasone, activate pregnane X receptor and upregulate the expression of *CYP3A* gene (Moore et al., 2000; Xie et al., 2000).

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 (Jacob et al., 1997). 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 (Fukui et al., 1991). LPS has been used extensively as a model of sepsis to study the inhibitory effects of inflammation and infection on CYP activity and expression (Li-Masters and Morgan, 2001; Pan et al., 2003). LPS-induced downregulation of *pregnane X receptor* and its target gene *cyp3a11* has been well characterized in mouse liver, placenta and fetal liver (Sewer et al., 1998; Beigneux et al., 2002; Sachdeva et al., 2003; Xu et al., 2004; Chen et al., 2005; Xu et al., 2005a,b). However, whether LPS downregulates the expressions of intestinal *pregnane X receptor* and its target gene *cyp3a11* remains to be elucidated.

In the present study, we investigated the in vivo effects of LPS on the expressions of *pregnane X receptor* and its target gene *cyp3a11* in mouse intestine. Our results indicate that LPS downregulated the expressions of *pregnane X receptor* and *cyp3a11* in mouse intestine. LPS-induced downregulation of intestinal *pregnane X receptor* and *cyp3a11* is mediated, at least in part, by oxidative stress.

2. Materials and methods

2.1. Chemicals

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

2.2. Animals and treatments

Female 8- to 10-week-old ICR mice, weighing 20–22 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/dark cycle in a controlled temperature (20–25 °C) and humidity (50±5%) environment for a period of 1 week before use.

To investigate the effects of LPS on the expressions of *pregnane X receptor*, *retinoid X receptor alpha* and the constitutive *cyp3a11* mRNA, all mice except controls received an intraperitoneal injection of LPS (0.1–5.0 mg/kg). Mice were sacrificed at 12 h after LPS treatment. Mouse proximal small intestines were excised for total RNA extraction.

To investigate the effects of LPS on the dexamethasone-inducible *cyp3a11* mRNA and erythromycin *N*-demethylase (ERND) activity, all groups except control were administered with dexamethasone (50 mg/kg, i.g.) for 3 days before LPS (1 mg/kg, i.p.) treatment. On the fourth day, mice were treated with dexamethasone (50 mg/kg, i.g.) plus LPS (1.0 mg/kg, i.p.) or saline. All mice from the controls were injected with saline. Mice were sacrificed at 24 h after LPS treatment. Mouse proximal small intestines were excised for total RNA extraction and microsome isolation.

To investigate the effects of *N*-acetylcysteine and ascorbic acid on LPS-induced downregulation of *pregnane X receptor*, *retinoid X receptor alpha* and the constitutive *cyp3a11* mRNA, mice were injected with LPS (1.0 mg/kg, i.p.), followed by an oral administration of *N*-acetylcysteine (150 mg/kg) or ascorbic acid (400 mg/kg) immediately after LPS treatment. Mice from the controls were orally administered with either saline, *N*-acetylcysteine or ascorbic acid. Mice were sacrificed at 12 h after LPS treatment. Mouse intestines were excised for total RNA extraction.

To investigate the effects of *N*-acetylcysteine and ascorbic acid on LPS-induced downregulation of the dexamethasone-inducible *cyp3a11* mRNA and ERND activity, all groups except control were orally administered with dexamethasone (50 mg/kg) for 3 days. On the fourth day, mice were administered with dexamethasone (50 mg/kg, i.g.) plus LPS (1.0 mg/kg, i.p), followed by an oral administration of *N*-acetylcysteine (150 mg/kg) or ascorbic acid (400 mg/kg) immediately after LPS treatment. Mice were sacrificed at 24 h after LPS treatment. Mouse proximal small intestines were excised for total RNA extraction and microsome isolation.

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

After laparotomy, mouse proximal small intestines were removed and washed with sterile ice-cold isotonic saline. Mucosae were scraped with a slide glass, on ice, in sterile conditions. Total cellular RNA was extracted from mucosa 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 were 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, 2.0 μg of total RNA 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, 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, 4 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 (Xu et al., 2004; Yang et al., 1999). *GAPDH*, 5′-GAG GGG CCA TCC ACA GTC TTC-3′ and 5′-CAT CAC CAT CTT CCA GGA GCG-3′; *pregnane X receptor*, 5′-GCG CGG AGA AGA CGG CAG CAT C-3′ and 5′-CCC AGG TTC CCG TTT CCG TGT C-3′; *retinoid X receptor αlpha*, 5′-GAG GCA AAC ATG GGG CTG AA-3′ and 5′-TGT CCC TGC CCT TTC TGG AT-3′; *cyp3a11*, 5′-CTC AAT GGT GTG TAT ATC CCC-3′ and 5′-CCG ATG TTC TTA GAC ACT GCC-3′. The sizes of amplified PCR products were 340 bp for *GAPDH*, 254 for *pregnane X receptor*, 721 for *retinoid X receptor αlpha* and 423 bp for *cyp3a11*, respectively. Number of cycles and annealing temperature were optimized for each primer pair. For *GAPDH*, 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 *pregnane X receptor* and *retinoid X receptor αlpha*, amplification was initiated by 3 min of denaturation at 94 °C for 1 cycle, followed by 45 cycles at 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min. After the last cycle of amplification, samples were incubated for 10 min at 72 °C. In preliminary experiments, *pregnane X receptor* and *retinoid X receptor αlpha* for 45 cycles were shown to lie in the linear portion of the curve for the amount of PCR products. For *cyp3a11*, number of cycles was distinguished between the constitutive and inducible expressions. For the constitutive expression of *cyp3a11*, amplification was initiated by 3 min of denaturation at 94 °C for 1 cycle, followed by 35 cycles at 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min. For the inducible expression of *cyp3a11*, amplification was initiated by 3 min of denaturation at 94 °C for 1 cycle, followed by 32 cycles at 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min. In preliminary experiments, the constitutive expression of *cyp3a11* for 35 cycles and the inducible expression for 32 cycles were shown to lie in the linear portion of the curve for the amount of PCR products. 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, St. Louis, MO) 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, St. Louis, MO) TBE buffer.

2.5. Preparation of intestinal microsomes

Intestinal microsomes were prepared as described elsewhere (Veau et al., 2002). Briefly, after laparotomy, mouse

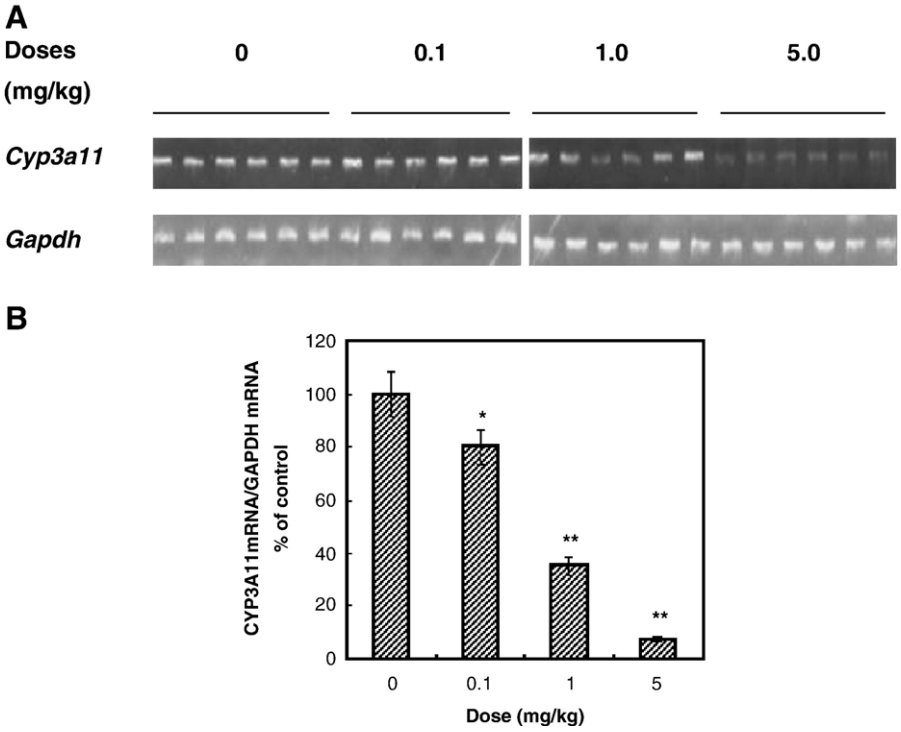

Fig. 1. LPS-induced downregulation of *cyp3a11* mRNA levels in mouse intestine. Mice were injected with different doses of LPS (0.1–5.0 mg/kg, i.p.). Mouse intestines were removed and total RNA was extracted from intestinal mucosa at 12 h after LPS treatment. (A) *Cyp3a11* mRNA levels were determined using RT-PCR. (B) Quantitative analysis of *cyp3a11* mRNA levels on six individual mouse intestinal RNA samples at each point was performed. *Cyp3a11* mRNA levels were normalized to *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* mRNA level in the same samples. *Cyp3a11* mRNA levels of the control were assigned as 100%. The number of mice at each point is six. *$P<0.05$, **$P<0.01$ as compared with control group.

proximal small intestines were quickly removed and washed with ice-cold isotonic saline containing 1 mM phenylmethylsulfonyl fluoride (PMSF) as protease inhibitor. Mucosae were scraped on ice with a slide glass and homogenized using a glass Teflon potter (20 strokes) in a buffer containing 250 mM sucrose, 50 mM Tris–HCl pH 7.4, 1 mM PMSF, and 0.1 U/ml aprotinin. The homogenates were centrifuged 10 min at 3000*g*, and the supernatant was again centrifuged for 30 min at 15,000*g*. The pellets containing the crude membranes were resuspended in 0.5 ml of a buffer containing 50 mM mannitol, 50 mM Tris pH 7.4, 1 mM PMSF, and 0.1 U/ml aprotinin and stored at −80 °C until use. Protein concentrations of microsome samples were measured according to the method of Lowry et al. (1951), using bovine serum albumin as a standard.

2.6. CYP3A catalytic activity

ERND was used as an indicator of CYP3A catalytic activity in this study. ERND was measured according to the method of Werringloer (1978) with a 45-min incubation containing 4 mM erythromycin in the presence of 0.5 mM NADPH and 0.4 mg of microsomal protein in a total assay volume of 1 ml. The rate of formaldehyde formation was determined spectrophotometrically at 412 nm using the Nash reagent. Measurement for ERND catalytic activity was repeated twice for three separately prepared liver microsome samples.

2.7. Lipid peroxidation assay

Lipid peroxidation was quantified by measuring thiobarbituric acid-reactive substance as described previously (Ohkawa et al., 1979). Tissue was homogenized in 9 volumes of 50 mM Tris–HCl buffer (pH 7.4) containing 180 mM KCl, 10 mM 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.

2.8. Statistical analysis

The *pregnane X receptor*, *retinoid X receptor alpha* and *cyp3a11* mRNA levels were normalized to *GAPDH* mRNA

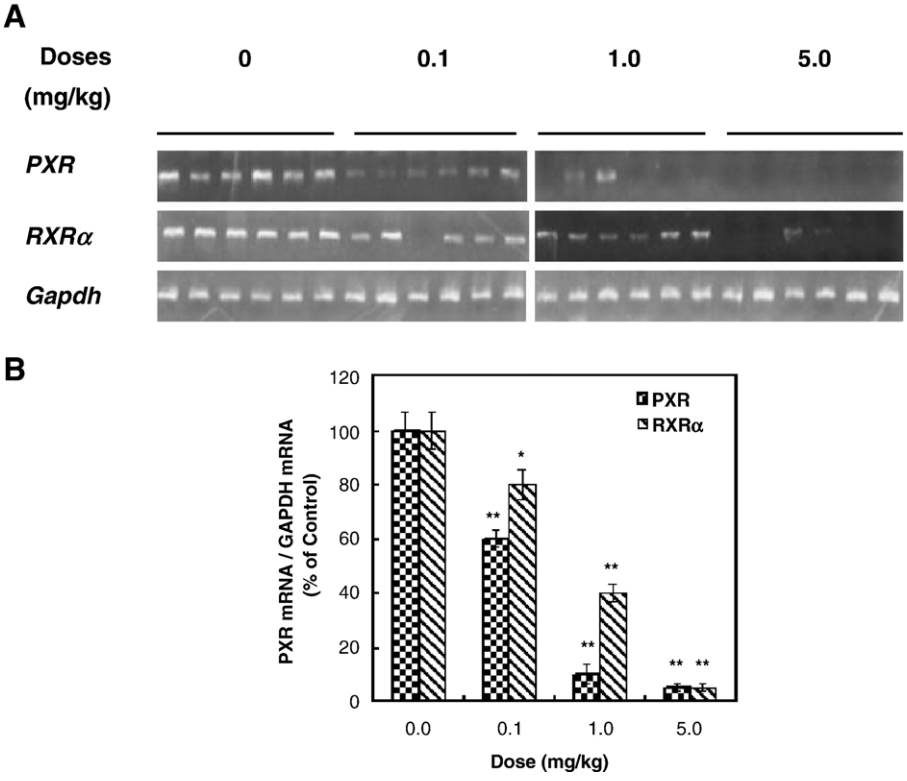

Fig. 2. LPS-induced downregulation of *pregnane X receptor* and *retinoid X receptor alpha* in mouse intestine. Mice were injected with different doses of LPS (0.1–5.0 mg/kg, i.p). Mouse intestines were removed and total RNA was extracted from intestinal mucosa at 12 h after LPS treatment. (A) *Pregnane X receptor (PXR)* and *retinoid X receptor alpha (RXRα)* mRNA levels were determined using RT-PCR. (B) Quantitative analysis of *pregnane X receptor (PXR)* and *retinoid X receptor alpha (RXRα)* mRNA levels on six individual mouse intestinal RNA samples at each point was performed. *Pregnane X receptor (PXR)* and *retinoid X receptor alpha (RXRα)* mRNA levels were normalized to *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* mRNA level in the same samples. *Pregnane X receptor (PXR)* and *retinoid X receptor alpha (RXRα)* mRNA levels of the control were assigned as 100%. The number of mice at each point is six. $^{**}P<0.01$ as compared with control group.

level in the same samples. *Pregnane X receptor*, *retinoid X receptor alpha* and *cyp3a11* mRNA levels of the control was assigned as 100%. Quantified data from analysis of RT-PCR and ERND assay were expressed as means ± S.E.M. at each point. A one-way analysis of variance (ANOVA) was used to determine differences between the treated animals and the control and statistical significance.

3. Results

3.1. Effects of LPS on the constitutive expressions of intestinal cyp3a11, pregnane X receptor and retinoid X receptor alpha

The effects of LPS on the constitutive expressions of *cyp3a11* mRNA are shown in Fig. 1. As expected, LPS treatment significantly decreased the level of intestinal *cyp3a11* mRNA in a dose-respondent manner. The effects of LPS on *pregnane X receptor* and *retinoid X receptor alpha* are presented in Fig. 2. Results showed that LPS significantly inhibited the expression of *pregnane X receptor* and its heterodimer *retinoid X receptor alpha* in mouse intestine in a dose-dependent manner.

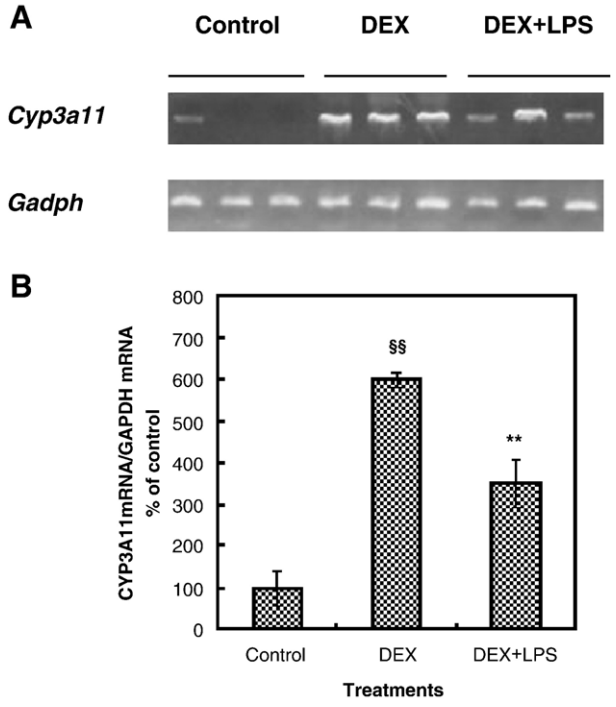

Fig. 3. The effects of LPS on the dexamethasone-inducible *cyp3a11* mRNA levels in mouse intestine. Mice were administered dexamethasone (DEX, 50 mg/kg, i.g.) for 3 days before LPS (1.0 mg/kg, i.p.) treatment. On the fourth day, mice were treated with dexamethasone (DEX, 50 mg/kg, i.g.) plus LPS (1.0 mg/kg, i.p). Mouse intestines were removed and total RNA was extracted from intestinal mucosa at 12 h after LPS treatment. (A) *Cyp3a11* mRNA levels were determined using RT-PCR. (B) *Cyp3a11* mRNA level on six individual mouse intestinal RNA samples at each point was analyzed quantitatively. *Cyp3a11* mRNA level was normalized to *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* mRNA level in the same samples. The *cyp3a11* mRNA level of control was assigned as 100%. $^{§§}P<0.01$ as compared with the control; $^{**}P<0.01$ as compared with DEX-treated group.

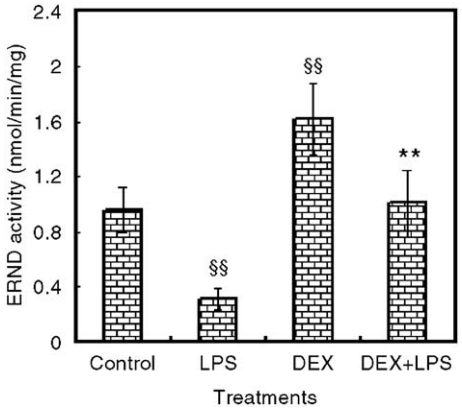

Fig. 4. The effects of LPS on the dexamethasone-inducible ERND catalytic activities in mouse intestine. Mice were administered dexamethasone (DEX, 50 mg/kg, i.g.) for 3 days. On the fourth day, mice were treated with dexamethasone (DEX, 50 mg/kg, i.g.) plus LPS (1.0 mg/kg, i.p). Mouse intestines were removed and microsomes were isolated from intestinal mucosa at 24 h after LPS treatment. ERND catalytic activities were measured as described in Materials and methods. Data were expressed as means ± S.E.M. of six mice. $^{§§}P<0.01$ as compared with the saline control; $^{**}P<0.01$ as compared with dexamethasone-treated group.

3.2. Effects of LPS on dexamethasone-inducible cyp3a11 mRNA and ERND catalytic activities

To investigate whether LPS treatment downregulates dexamethasone-inducible expression of *cyp3a11* mRNA, mice were pretreated with dexamethasone (50 mg/kg) for 3 days before LPS treatment. On the fourth day, mice were orally administered with dexamethasone (50 mg/kg) plus LPS (1.0 mg/kg, i.p.) or saline. The results are presented in Fig. 3. As expected, a 4-day pretreatment with dexamethasone resulted in a six-fold increase in *cyp3a11* mRNA in mouse intestine. LPS treatment decreased intestinal *cyp3a11* mRNA by about 50% in mice pretreated with dexamethasone. The effects of LPS

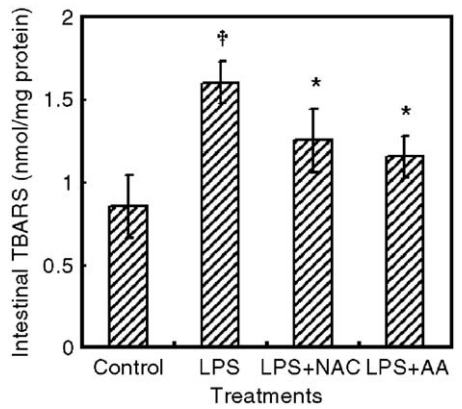

Fig. 5. The effects of *N*-acetylcysteine and ascorbic acid on LPS-induced *lipid peroxidation* in mouse intestine. Mice were then injected with LPS (1.0 mg/kg, i.p.), followed by single dose of either *N*-acetylcysteine (NAC, 150 mg/kg, i.g.) or ascorbic acid (AA, 400 mg/kg, i.g.) immediately after LPS treatment. Intestine was excised and homogenized at 12 h after LPS treatment. Thiobarbituric acid-reactive substance (TBARS) content was measured as described in Materials and methods. Data were expressed as means ± S.E.M. ($n=9$). $^{††}P<0.01$ as compared with control group. $^{**}P<0.01$ as compared with LPS-treated group.

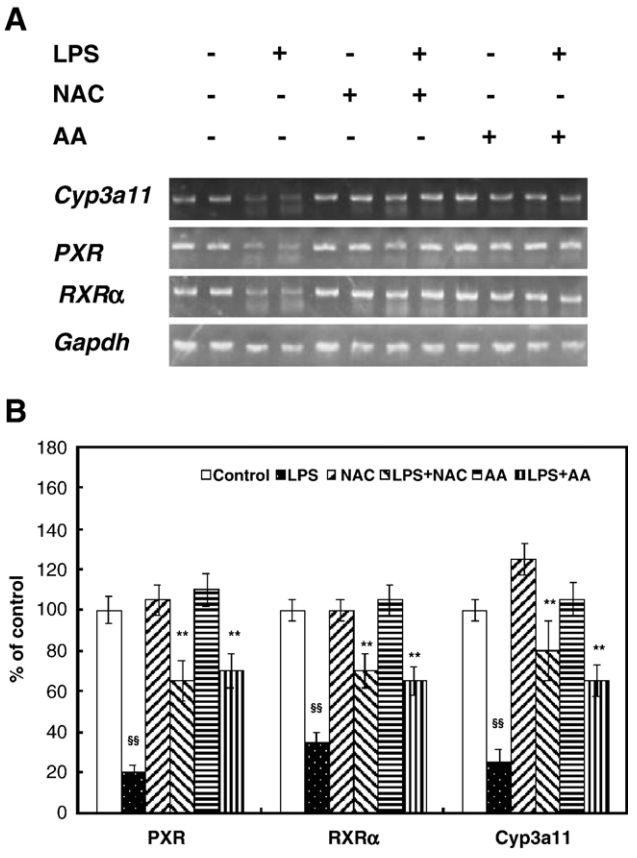

Fig. 6. The effects of *N*-acetylcysteine and ascorbic acid on LPS-induced downregulation of *pregnane X receptor*, *retinoid X receptor alpha* and *cyp3a11*. Mice were then injected with LPS (1.0 mg/kg, i.p.), followed by single dose of either *N*-acetylcysteine (NAC, 150 mg/kg, i.g.) or ascorbic acid (AA, 400 mg/kg, i.g.) immediately after LPS treatment. Mouse intestines were removed and total RNA was extracted from intestinal mucosa at 12 h after LPS treatment. (A) *Pregnane X receptor (PXR)*, *retinoid X receptor alpha (RXRα)* and *cyp3a11* mRNA were determined using RT-PCR. (B) Quantitative analysis of *pregnane X receptor (PXR)*, *retinoid X receptor alpha (RXRα)* and *cyp3a11* mRNA on six individual mouse liver RNA samples at each point was performed. *Pregnane X receptor (PXR)*, *retinoid X receptor alpha (RXRα)* and *cyp3a11* mRNA levels were normalized to *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* mRNA level in the same samples. *Pregnane X receptor (PXR), retinoid X receptor alpha (RXRα)* and *cyp3a11* mRNA levels of control were assigned as 100%. $^{§§}P<0.01$ as compared with the saline control; $^{**}P<0.01$ as compared with LPS-treated group.

treatment on dexamethasone-inducible ERND catalytic activity are presented in Fig. 4. Results showed that dexamethasone alone caused about 2-fold induction on ERND catalytic activity. LPS significantly inhibited intestinal ERND catalytic activity in mice pretreated with dexamethasone.

3.3. Effects of LPS on intestinal lipid peroxidation

In the present study, thiobarbituric acid-reactive substance was used as a remark of *lipid peroxidation*. The effects of LPS on intestinal thiobarbituric acid-reactive substance are presented in Fig. 5. Results showed that LPS treatment significantly increased the level of intestinal thiobarbituric acid-reactive substance. *N*-acetylcysteine and ascorbic acid significantly attenuated LPS-induced *lipid peroxidation*.

3.4. Effects of N-acetylcysteine and ascorbic acid on LPS-induced downregulation of intestinal cyp3a11, pregnane X receptor and retinoid X receptor alpha

The effects of *N*-acetylcysteine and ascorbic acid on LPS-induced downregulation of the constitutive expressions of intestinal *cyp3a11*, *pregnane X receptor* and *retinoid X receptor αlpha* mRNA are presented in Fig. 6. Results showed that oral administration with either *N*-acetylcysteine or ascorbic acid significantly attenuated LPS-induced downregulation of intestinal *cyp3a11*, *pregnane X receptor* and *retinoid X receptor αlpha* mRNA. The effects of *N*-acetylcysteine and ascorbic acid on LPS-induced downregulation of dexamethasone-inducible *cyp3a11* mRNA and ERND catalytic activity were analyzed. The results are presented in Figs. 7 and 8. As expected, pretreatment with either *N*-acetylcysteine or ascorbic acid partially attenuated LPS-induced downregulation of *cyp3a11* mRNA and ERND catalytic activity in mouse intestine.

4. Discussion

CYP3A is the principal enzymes involved in the metabolism of drugs in intestine. Recent studies indicate that LPS-induced inflammation imposes a reduction in the intestinal CYP3A expression and activity in rats (Kalitsky-Szirtes et al., 2004). In the present study, we investigated the effects of LPS treatment on the constitutive expression of *cyp3a11* mRNA in mouse intestine. Our results found that LPS treatment significantly decreased the level of intestinal *cyp3a11* mRNA in a dose-respondent manner. These results are in agreement with earlier

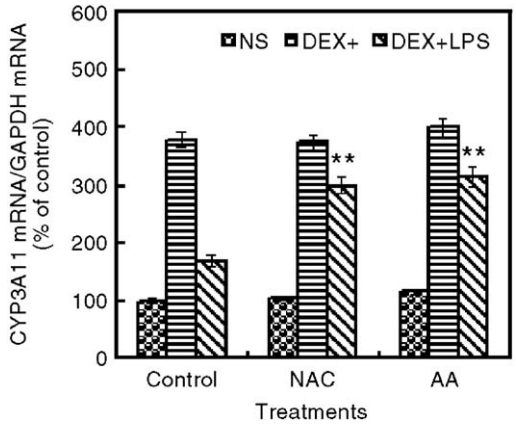

Fig. 7. The effects of *N*-acetylcysteine and ascorbic acid on LPS-induced downregulation of the dexamethasone-inducible *Cyp3a11* mRNA. Mice were administered dexamethasone (DEX, 50 mg/kg, i.g.) for 3 days. On the fourth day, mice were administered dexamethasone (DEX, 50 mg/kg, i.g.) plus LPS (1.0 mg/kg, i.p.), followed by a single dose of *N*-acetylcysteine (NAC, 150 mg/kg, i.g.) or ascorbic acid (AA, 400 mg/kg, i.g.) immediately after LPS treatment. Mouse intestines were removed and total RNA was extracted from intestinal mucosa at 12 h after LPS treatment. *Cyp3a11* mRNA levels on six individual mouse liver RNA samples at each point were analyzed quantitatively. *Cyp3a11* mRNA level was normalized to *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* mRNA level in the same samples. The *cyp3a11* mRNA level of control was assigned as 100%. $^{**}P<0.01$ as compared with LPS+DEX-cotreated group.

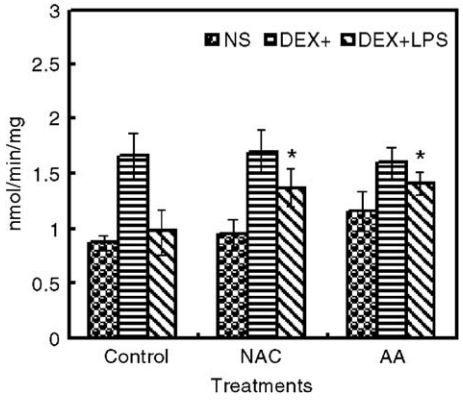

Fig. 8. The effects of *N*-acetylcysteine and ascorbic acid on LPS-induced downregulation of the inducible ERND catalytic activities. Mice were administered with dexamethasone (DEX, 50 mg/kg, i.g.) for 3 days. On the fourth day, mice were administered with dexamethasone (DEX, 50 mg/kg, i.g.) plus LPS (1.0 mg/kg, i.p.), followed by a single dose of *N*-acetylcysteine (NAC, 150 mg/kg, i.g.) or ascorbic acid (AA, 400 mg/kg, i.g.) immediately after LPS treatment. Mouse intestines were removed and microsomes were isolated from intestinal mucosa at 24 h after LPS treatment. ERND catalytic activities were measured as described in Materials and methods. Data were expressed as means±S.E.M. of six mice. *$P<0.05$ as compared with DEX+LPS-cotreated group.

studies by others, in which LPS significantly downregulated the expression of *cyp3a11* in mouse liver (Sewer et al., 1998; Beigneux et al., 2002; Sachdeva et al., 2003; Xu et al., 2004).

The pregnane X receptor is a member of the nuclear receptor superfamily that regulates *cyp3a11* transcription in mouse liver in a ligand-dependent manner. Several lines of evidence suggest that pregnane X receptor is also the key regulator of *cyp3a11* gene expression in mouse intestine. First, *pregnane X receptor* is highly expressed in intestine, where *cyp3a11* genes are most highly expressed and induced (Kliewer et al., 1998). Second, *cyp3a11* was induced by pregnenolone 16-alpha-carbonitrile in small intestine of wild-type mice, while no regulation of *cyp3a11* was observed in *pregnane X receptor*-null mice (Maglich et al., 2002). Previous studies demonstrated that LPS treatment downregulated the expression of hepatic *pregnane X receptor* mRNA (Xu et al., 2004). LPS-induced downregulation of hepatic *cyp3a11* is associated with repression of *pregnane X receptor* during the acute phase response (Beigneux et al., 2002; Sachdeva et al., 2003). In the present study, we sought to determine whether LPS downregulates the expression of *pregnane X receptor* mRNA in mouse intestine. We found that LPS significantly inhibited the expression of *pregnane X receptor* and its heterodimer *retinoid X receptor alpha* in mouse intestine in a dose-dependent manner.

A recent report indicated that pretreatment with dexamethasone increased the expression of CYP3A in intestinal microsomes of rats by approximately 5-fold (Lin et al., 1999). The present study showed that a 4-day pretreatment with dexamethasone resulted in a 6-fold increase in *cyp3a11* mRNA in mouse intestine. Interestingly, *cyp3a11* mRNA was decreased by about 50% in mice pretreated with dexamethasone plus LPS as compared with mice pretreated with dexamethasone alone, suggesting that LPS treatment downregulates dexamethasone-inducible expression of *cyp3a11* mRNA. It has been demonstrated that mouse CYP3A is the major enzyme catalyzing erythromycin *N*-demethylation (Lan et al., 2000). Thus, ERND activity was used as an indicator of CYP3A expression in this study. The present study found that dexamethasone alone caused about 2-fold induction on ERND catalytic activity. LPS significantly inhibited intestinal ERND catalytic activity in mice pretreated with dexamethasone.

During sepsis, the production of reactive oxygen species, such as nitric oxide and superoxide, increases in small intestine. The generation of these free radical species can contribute to nucleus factor-kappa B (NF-κB) activation and promote intestinal lipid peroxidation and subsequent tissue injury (Mercer et al., 1996; Salvemini et al., 1999; Lush et al., 2003). On the other hand, reactive oxygen species have been associated with the expression of a number of genes and signal transduction pathways (Allen, 1998; Allen and Tresini, 2000). Our earlier study found that reactive oxygen species contribute to LPS-induced downregulation of *pregnane X receptor* and its target gene *cyp3a11* in mouse liver (Xu et al., 2004, 2005a,b). Therefore, whether LPS-induced downregulation of intestinal *pregnane X receptor* and *cyp3a11* is associated with oxidative stress is especially interesting. The present study found that LPS treatment significantly increased the level of intestinal thiobarbituric acid-reactive substance, a remark of *lipid peroxidation*. These results are in agreement with earlier work of Memon et al. (2000), in which serum thiobarbituric acid-reactive substance are increased by LPS treatment. To determine the role of oxidative stress in LPS-induced downregulation of intestinal *pregnane X receptor* and its target gene *cyp3a11*, *N*-acetylcysteine and ascorbic acid, two well-known antioxidants, were orally administered to counteract LPS-induced oxidative stress in mouse intestine. As expected, *N*-acetylcysteine and ascorbic acid significantly attenuated LPS-induced *lipid peroxidation*. Correspondingly, LPS-induced downregulation of intestinal *pregnane X receptor* and *retinoid X receptor αlpha* mRNA was significantly attenuated in mice administered orally with either *N*-acetylcysteine or ascorbic acid. Furthermore, pretreatment with either *N*-acetylcysteine or ascorbic acid partially attenuated LPS-induced downregulation on the constitutive and dexamethasone-inducible expressions of *cyp3a11* mRNA and ERND catalytic activity in mouse intestine. These results suggest that oxidative stress may be involved in LPS-induced downregulation of intestinal *pregnane X receptor* and its target gene *cyp3a11*.

LPS stimulates tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and interleukin-1β (IL-1β) production in mucosa of the small intestine (Meyer et al., 1995; Ulich et al., 1990). Numerous studies demonstrated that proinflammatory cytokines, such as TNF-α, IL-1β and IL-6, were involved in LPS-induced downregulation of *cytochrome P450s* in liver (Sewer and Morgan, 1997; Monshouwer et al., 1996; Ashino et al., 2004; Miyoshi et al., 2005). An in vitro study found that TNF-α markedly suppressed the expression of *CYP3A4* in human colon carcinoma HT-29 cells (Chun et al., 2002).

Therefore, the present results do not exclude the involvement of proinflammatory cytokines in LPS-induced downregulation of *pregnane X receptor* and *cyp3a11* in mouse intestine.

In summary, the present results allow us to reach the following conclusions. First, LPS represses the expressions of intestinal *cyp3a11*; second, LPS-induced repression of intestinal *cyp3a11* is associated with downregulation of *pregnane X receptor* and *retinoid X receptor αlpha* in mouse intestine; third, LPS-induced downregulation of *pregnane X receptor* and its target gene *cyp3a11* in mouse intestine is mediated, at least in part, by oxidative stress.

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