Free Radical Biology & Medicine, Vol. 37, No. 1, pp. 10–22, 2004

0891-5849/$-see front matter

ELSEVIER

doi:10.1016/j.freeradbiomed.2004.03.021

Original Contribution

KUPFFER CELLS AND REACTIVE OXYGEN SPECIES PARTIALLY MEDIATE LIPOPOLYSACCHARIDE-INDUCED DOWNREGULATION OF NUCLEAR RECEPTOR PREGNANE X RECEPTOR AND ITS TARGET GENE *CYP3A* IN MOUSE LIVER

De-Xiang Xu,*,† Wei Wei,* Mei-Fang Sun,† Cheng-Yi Wu,* Jian-Ping Wang,† Ling-Zhen Wei,† and Cheng-fan Zhou†

*Institute of Clinical Pharmacology, Anhui Medical University, Hefei, 230032, People's Republic of China
†Department of Toxicology, Anhui Medical University, Hefei, 230032, People's Republic of China

(*Received* 12 *November* 2003; *Revised* 22 *March* 2004; *Accepted* 26 *March* 2004)

Available online 23 April 2004

Abstract—Pregnane X receptor (PXR) is a member of the nuclear receptor superfamily that regulates target gene transcription in a ligand-dependent manner. The in vivo effects of lipopolysaccharide (LPS) on expression of *PXR* and its target gene *cytochrome P450 3A* (*CYP3A*) in mouse liver were investigated in this study. Mice were injected intraperitoneally with different doses of LPS (0.1–5.0 mg/kg). *PXR* and *CYP3A11* mRNA levels were measured using reverse transcription polymerase chain reaction. Results indicate that LPS significantly inhibits the expression of *PXR* mRNA in a dose-dependent manner, followed by suppression of *CYP3A11* mRNA in mouse liver. LPS also represses the upregulation of *CYP3A11* mRNA levels and erythromycin *N*-demethylase (ERND) catalytic activity in mice pretreated with PXR ligands dexamethasone, rifampicin, mifepristone, and phenobarbital. LPS-induced downregulation of *PXR* and *CYP3A11* mRNA in liver was significantly attenuated in mice pretreated with gadolinium chloride, a selective Kupffer cell toxicant. Pretreatment with a single dose of gadolinium chloride (10 mg/kg) also significantly attenuated LPS-induced downregulation of dexamethasone-, rifampicim-, mifepristone-, and phenobarbital-inducible, *CYP3A11* mRNA expression and ERND activity in mouse liver. Furthermore, LPS-induced downregulation of *PXR* and *CYP3A11* mRNA was significantly attenuated in mice pretreated with allopurinol, an inhibitor of xanthine oxidase, and diphenyleneiodonium chloride, an inhibitor of NADPH oxidase. Allopurinol and diphenyleneiodonium chloride pretreatment also attenuated the repressive effects of LPS on dexamethasone-, rifampicin-, mifepristone-, and phenobarbital-inducible *CYP3A11* mRNA expression and ERND catalytic activity in mouse liver. However, aminoguanidine, a selective inhibitor of inducible nitric oxide synthase, has no effect on LPS-induced downregulation of *PXR* and *CYP3A11* mRNA. Finally, LPS-induced downregulation of PXR and *CYP3A11* mRNA was prevented in mice pretreated with either *N*-acetylcysteine or ascorbic acid. These antioxidants also prevented the repressive effects of LPS on dexamethasone-, rifampicin-, mifepristone-, and phenobarbital-inducible *CYP3A11* mRNA expression and ERND catalytic activity in mouse liver. These results indicate that Kupffer cells contribute to LPS-induced downregulation of *PXR* and *CYP3A* in mouse liver. Reactive oxygen species, produced possibly by NADPH oxidase and perhaps by xanthine oxidase, are involved in LPS-induced downregulation of nuclear receptor PXR and its target gene *CYP3A* in mouse liver.

Keywords—Lipopolysaccharide, Pregnane X receptor, *Cytochrome P450 3A*, Kupffer cells, Reactive oxygen species, Antioxidants, Free radicals

Address correspondence to: Professor Wei Wei, Institute of Clinical Pharmacology, Anhui Medical University, Hefei, 230032, People's Republic of China; Fax: +86 551 5161208; E-mail: wwei@ahmu.edu.cn; Prof. De-Xiang Xu, Department of Toxicology, Anhui Medical University, Hefei 230032, People's Republic of China; Fax: +86 551 5161168; E-mail: xudex@mail.hf.ah.cn.

INTRODUCTION

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 [1]. *CYP3A* expression in liver is

highly regulated by development, tissue-specific factors, hormonal influences, xenobiotics, and pathophysiological mechanisms [2,3]. Recent studies have reported that pregnane X receptor (*PXR*), a member of the nuclear receptor superfamily, regulates *CYP3A* gene transcription in a ligand-dependent manner [4–6]. *CYP3A* inducers, such as dexamethasone, rifampicin, mifepristone, and phenobarbital, activate PXR and upregulate expression of the *CYP3A* gene [7,8].

On the other hand, numerous studies have indicated that inflammation and infection reduce cytochrome P450 levels in various species including human, rat, and mouse [9,10]. Lipopolysaccharide (LPS)-induced downregulation of *CYP3A* has also been demonstrated in a mouse model [11,12]. Furthermore, recent studies have shown that LPS-induced downregulation of *CYP3A* is associated with a marked reduction in *PXR* mRNA and protein levels [13,14].

In vivo and in vitro studies have shown that pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α, as well as IFN, can mimic the downregulation of P450 gene products seen during infection or inflammation [15–17], so pro-inflammatory cytokines might be responsible for LPS-induced downregulation of cytochrome P450 gene expression. Moreover, Kupffer cells are the resident macrophages of the liver. When activated by LPS, Kupffer cells produce and release numerous mediators, including reactive oxygen species (ROS) and pro-inflammatory cytokines [18]. Therefore, Kupffer cells may play an important role in LPS-induced downregulation of *PXR* and *CYP3A*.

On the other hand, Warren et al. [19] tested the role of TNF-α in P450 downregulation by LPS in mice, using animals deficient in both the p55 and p75 receptors for this cytokine. The results indicated that LPS caused similar decreases in hepatic microsomal CYP1A, -2B, -3A, and -4A proteins and activities in both wild-type and TNF-α receptor-deficient animals. Siewert et al. [20] also observed a similar lack of effect of IL-6 gene deletion on suppression of *CYP1A2, -2A5, -2E1, and -3A11* mRNAs after LPS administration to mice. These results suggest that multiple cytokines released during LPS-induced inflammation are involved in down-regulation of cytochrome P450. Moreover, ROS are known to influence the expression of a number of genes, including pro-inflammatory cytokines. Therefore, we hypothesized that ROS may be involved in LPS-induced downregulation of nuclear receptor PXR and its target gene *CYP3A* in mouse liver.

In present study, we investigated the in vivo effects of LPS on *PXR* and *CYP3A* expression in mouse liver. Our results found that Kupffer cells and ROS mediate, at least partly, the LPS-induced downregulation of PXR and *CYP3A* in mouse liver.

MATERIALS AND METHODS

Chemicals

Lipopolysaccharide (*Escherichia coli* LPS, serotype 0127:B8), dexamethasone (Dex), rifampicin (RIF), mifepristone (RU486), phenobarbital (PB), gadolinium chloride ($GdCl_3 \cdot 6H_2O_2$), allopurinol (ALL), diphenyleneiodonium chloride (DPI), aminoguanidine (AG), ascorbic acid (AA), and *N*-acetylcysteine (NAC) were purchased from Sigma Chemical Company (St. Louis, MO, USA).

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 1 week before use. The present study comprised several separate experiments. The doses of $GdCl_3$, allopurinol, DPI, AG, NAC, and AA used in the present study were referred to others [21–25].

Experiment 1. To investigate the effects of LPS on the constitutive *PXR* and *CYP3A11* mRNAs, all mice except controls received a single of LPS (0.1–5.0 mg/kg, i.p.). To investigate the effects of LPS on inducible *CYP3A11* mRNA and erythromycin *N*-demethylase (ERND) activity, all groups except the control were administered Dex (40 mg/kg, i.p.), RIF (50 mg/kg, i.g.), RU486 (50 mg/kg, s.c.), or PB (75mg/kg, i.p.) for 3 days. On the fourth day, mice were treated with LPS (1.0 mg/kg, i.p.) or saline. Two hours later, mice were administered Dex (40 mg/kg, i.p.), RIF (50 mg/kg, i.g.), RU486 (50 mg/kg, s.c.), or PB (75 mg/kg, i.p.). All control mice were injected with saline. Mice were sacrificed 12 and 18 h after LPS treatment. Mouse livers were excised for total RNA extraction and microsome isolation.

Experiment 2. To investigate the effects of $GdCl_3$ on *TNF-α*, *IL-1β*, *IL-6, PXR,* and *CYP3A11* mRNA expression, mice were administered a single dose of $GdCl_3$ (10 mg/kg, i.v.), followed by an intraperitoneal injection of LPS (1.0 mg/kg) 24 h after $GdCl_3$ treatment. Control mice (saline and $GdCl_3$) were administered either saline or $GdCl_3$ (10 mg/kg, i.v.). Mice were sacrificed 1, 3, 6, and 12 h after LPS treatment. Mouse livers were excised for total RNA extraction. To investigate the effects of $GdCl_3$ on LPS-induced downregulation of inducible *CYP3A11* mRNA and ERND activity, all groups except the control were

administered Dex (40 mg/kg, i.p.), RIF (50 mg/kg, i.g.), RU486 (50 mg/kg, s.c.), or PB (75 mg/kg, i.p.) for 3 days. On the third day, mice were administered a single dose of $GdCl_3$ (10 mg/kg, i.v.). On the fourth day, mice were treated with LPS (1.0 mg/kg, i.p.) or saline. Two hours later, mice were administered Dex (40 mg/kg, i.p.), RIF (50 mg/kg,i.g.), RU486 (50 mg/kg, s.c.), or PB (75 mg/kg, i.p.). Mice were sacrificed 12 and 18 h after LPS treatment. Mouse livers were excised for total RNA extraction and microsome isolation.

Experiment 3. To investigate the effects of ALL, DPI, and AG on LPS-induced downregulation of *PXR* and *CYP3A11* mRNAs, mice were administered ALL (100 mg/kg, i.g.), DPI (1.0 mg/kg, s.c.), or AG (150 mg/kg, i.p.) 48, 24, and 2 h before LPS treatment. Mice were then administered LPS (1.0 mg/kg, i.p.), followed by two doses of ALL (100 mg/kg, i.g.), DPI (1.0 mg/kg, s.c.), or AG (150 mg/kg, i.p.) 2 and 6 h after LPS treatment. Control mice were administered saline, ALL (100 mg/kg, i.g.), DPI (1.0 mg/kg, s.c.), or AG (150 mg/kg, i.p.). Mice were sacrificed 12 h after LPS treatment. Mouse livers were excised for total RNA extraction. To investigate the effects of ALL, DPI, and AG on LPS-induced downregulation of inducible CYP3A11 mRNA and ERND activity, all groups except control were administered Dex (40 mg/kg, i.p.), RIF (50 mg/kg, i.g.), RU486 (50 mg/kg, s.c.), or PB (75mg/kg, i.p.) for 3 days before LPS treatment. Mice were administered ALL (100 mg/kg, i.g.), DPI (1.0 mg/kg, s.c.), or AG (150 mg/kg, i.p.) 48, 24, and 2 h before LPS treatment. On the fourth day, mice were injected with LPS (1.0 mg/kg, i.p.) and CYP3A inducers, followed by two doses of ALL (100 mg/kg, i.g.), DPI (1.0 mg/kg, s.c.), or AG (150 mg/kg, i.p.) 2 and 6 h after LPS treatment. Mice were sacrificed 12 and 18 h after LPS treatment. Mouse livers were excised for total RNA extraction and microsome isolation.

Experiment 4. To investigate the effects of NAC and AA on LPS-induced downregulation of PXR and CYP3A11 mRNAs, mice were administered NAC (150 mg/kg, i.v.), or AA (400 mg/kg, i.v.) 48, 24 and 2 h before LPS treatment. Mice were then injected with LPS (1.0 mg/kg, i.p.), followed by two doses of NAC (150 mg/kg, i.v.) or ascorbic acid (400 mg/kg, i.v.) 2 and 6 h after LPS treatment. Control mice were administered either saline, NAC, or AA. Mice were sacrificed 12 h after LPS treatment. Mouse livers were excised for total RNA extraction. To investigate the effects of NAC and AA on LPS-induced downregulation of Dex-inducible CYP3A11 mRNA and ERND activity, all groups except the control were administered Dex (40 mg/kg, i.p.), RIF (50 mg/kg, i.g.), RU486 (50 mg/kg, s.c.), or PB (75 mg/kg, i.p.) for 3 days before LPS (1 mg/kg i.p.) treatment. Mice were pretreated with NAC (150 mg/kg, i.v.) or AA (400 mg/kg, i.v.) 48, 24, and 2 h before LPS administration. On the fourth day, mice were administered LPS (1.0 mg/kg, i.p.) and CYP3A inducer, followed by two doses of NAC (150 mg/kg, i.v.) or AA (400 mg/kg, i.v.) 2 and 6 h after LPS treatment. Mice were sacrificed 12 and 18 h after LPS treatment. Mouse livers 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.

Analysis of plasma nitrite and nitrate concentration

Mice were administered AG (150 mg/kg, i.p.) 48, 24, and 2 h before LPS administration. Mice were then injected with LPS (1.0 mg/kg, i.p.), followed by repeated doses of AG at 4 h intervals. The stable end products of L-arginine-dependent nitric oxide synthesis, nitrate and nitrite, were measured in plasma using a colorimetric method based on the Griess reaction [26,27]. Briefly, aliquots of plasma were added to 35% sulfosalicylic acid and vortexed every 5 min for 30 min to deproteinize samples. The samples were then centrifuged at 10,000*g* at 4°C for 15 min. An aliquot of the supernatant was taken for nitrite and nitrate analysis. Twenty microliters of plasma sample was mixed with 20 μl of 0.31 M phosphate buffer, pH 7.5, 10 μl of 0.1 mM FAD, 10 μl of 1 mM NADPH, 10 ml of nitrate reductase (10 units/ml), and 30 μl of water in a 96-well plate. The reaction was allowed to proceed for 1 h in the dark. The percentage conversion of nitrate to nitrite was 98%. To each sample, 1 μl of lactate dehydrogenase (1500 units/ml) and 10 μl of 100 mM pyruvic acid were added and incubated for 15 min at 37°C. The samples were then mixed with an equivalent volume of Griess reagent and incubated for an additional 10 min at room temperature. Nitrite levels were determined colorimetrically at 550 nm with a Universal microplate reader (Bio-Tek Instruments, Inc.) and a sodium nitrite standard curve.

Isolation of total RNA and RT

Fifty milligrams of liver tissue was collected from each mouse. Total cellular RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), 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 reverse transcriptase (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.

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. The following primers were synthesized by Shanghai Sangon Biological Engineering Technology and Service Company (Shanghai, China), according to sequence designs previously described by others [28–31]. GAPDH, 5′-GAG GGG CCA TCC ACA GTC TTC-3′ and 5′-CAT CAC CAT CTT CCA GGA GCG-3′; 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

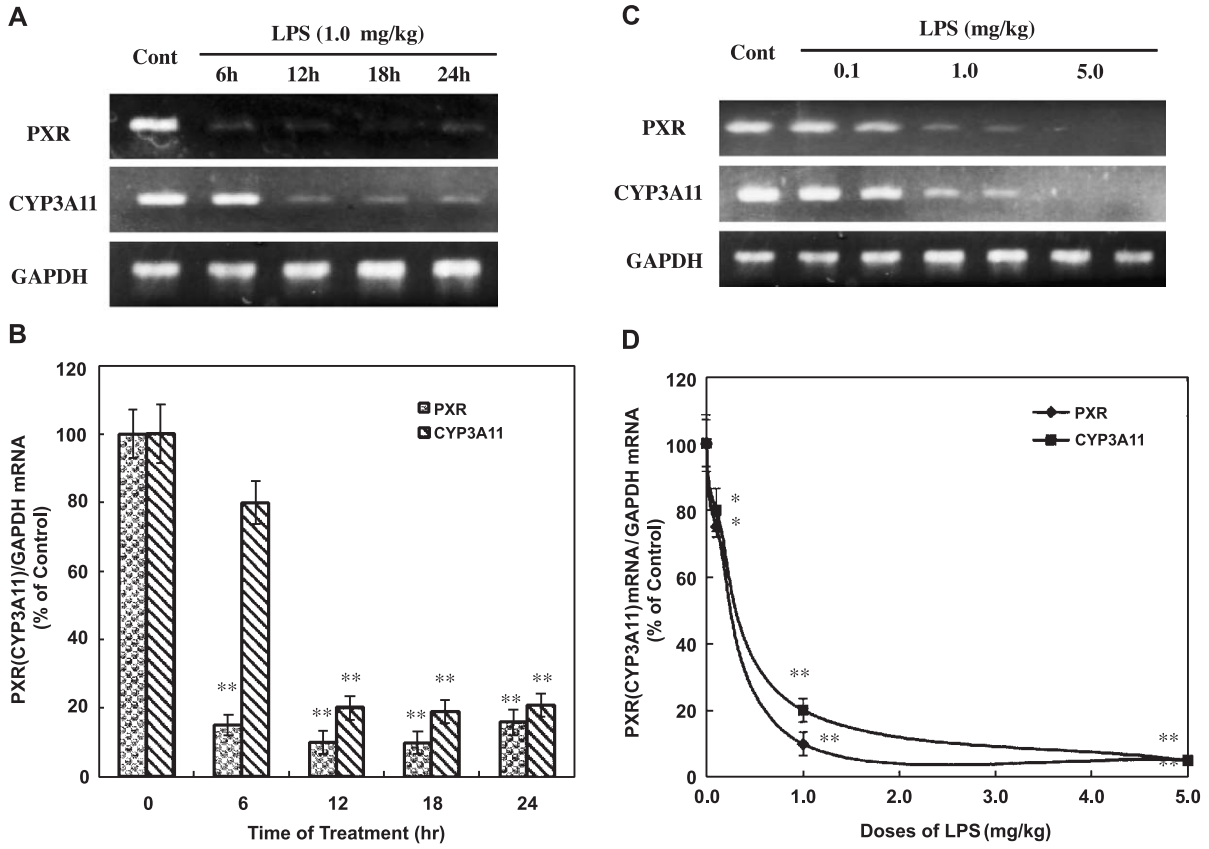

Fig. 1. Effects of LPS on *PXR* and *CYP3A11* mRNA levels in mouse liver. (A) Mice were injected with LPS (1.0 mg/kg, i.p.). Livers were excised and total RNA was extracted at 6, 12, 18, and 24 h after LPS treatment. Total RNA extracted from four mouse livers was pooled on each treatment. *PXR* and *CYP3A11* mRNA levels were determined using RT-PCR. (B) Quantitative analysis of *PXR* and *CYP3A11* mRNAs was performed on four individual mouse liver RNA samples at each point. *PXR* and *CYP3A11* mRNA levels were normalized to the *GAPDH* mRNA level in the same samples. The *PXR* and *CYP3A11* mRNA levels at 0 h were set at 100%. $^{**}p < .01$ as compared with 0 h group. (C) Mice were injected with different doses of LPS (0.1–5.0 mg/kg, i.p.). Livers were excised and total RNA was extracted 12 h after LPS treatment. Total RNA extracted from four mouse livers was pooled on each treatment. *PXR* and *CYP3A11* mRNA levels were determined using RT-PCR. (D) Quantitative analysis of *PXR* and *CYP3A11* mRNAs was performed on four individual mouse liver RNA samples at each point. *PXR* and *CYP3A11* mRNA levels were normalized to the *GAPDH* mRNA level in the same samples. *PXR* and *CYP3A11* mRNA levels of the control were set at as 100%. The number of mice at each point is 4. $^{*}p < .05$, $^{**}p < .01$ as compared with control group.

TTC GAG GCT CCA GTG AAT TCG G-3′; IL-1β, 5′-TCA TGG GAT GAT GAT GAT AAC CTG CT 3′ and 5′-CCC ATA CTT TAG GAA GAC ACG GAT T-3′; IL-6, 5′-CTG GTG ACA ACC ACG GCC TTC CCT A-3′, 5′-ATG CTT AGG CAT AAC GCA CTA GGT T-3′. The sizes of amplified PCR products were 340 bp for *GAPDH*, 254 for *PXR*, 423 bp for *CYP3A11*, 307 bp for TNF-α, 502 bp for IL-1β, and 600 bp for IL-6, 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 *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. After the last cycle of amplification, samples were incubated for 10 min at 72°C. In preliminary experiments, *PXR* for 45 cycles was 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 expression. For constitutive expression of *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 inducible expression of *CYP3A11*, amplification was initiated by 3 min of denaturation at 94°C for 1 cycle, followed by 25 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min. In preliminary experiments, constitutive expression of *CYP3A11* for 30 cycles and inducible expression for 45 cycles was shown to lie in the linear portion of the curve for the amount of PCR products. 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. pBR322 DNA digested with *Alu*I was used as a molecular marker (MBI Fermentas). Agarose gels were stained with 0.5 mg/ml ethidium bromide (Sigma) TBE buffer.

Preparation of liver microsomes

Microsomes were isolated from livers by differential centrifugation [32]. All procedures were conducted at 4°C. Tissue was homogenized in 4 vol of Tris–chloride buffer, pH 7.4, containing 150 mM potassium chloride and 1 mM EDTA, with a Polytron homogenizer and centrifuged at 10,000*g* for 20 min. The supernatant was collected and centrifuged at 211,000*g* for 40 min. The microsomal pellet was resuspended and washed in sodium pyrophosphate buffer, pH 7.4, containing 1 mM EDTA and centrifuged again at 211,000*g* for 40 min at 4°C. The washed microsomal

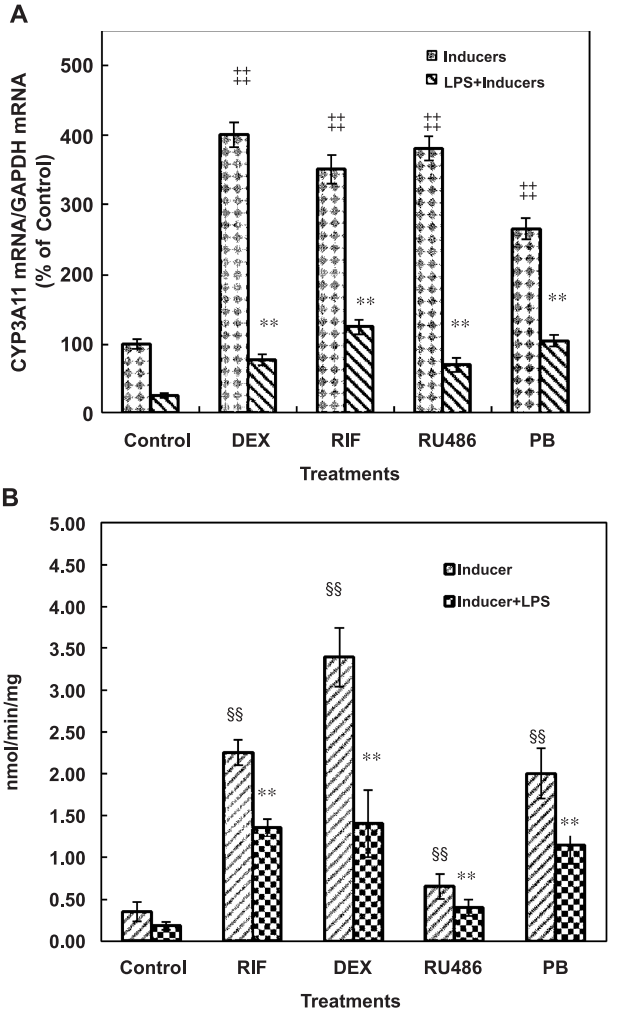

Fig. 2. Effects of LPS on inducible *CYP3A11* mRNA levels and ERND catalytic activity in mouse liver. Mice were administered Dex (40 mg/kg, i.p.), RIF (50 mg/kg, i.g.), RU486 (50 mg/kg, s.c.) or PB (75 mg/kg, i.p.) for 3 days before LPS (1 mg/kg i.p.) treatment. (A). Total RNA in mouse livers was extracted 12 h after LPS treatment. *CYP3A11* mRNA level was analyzed quantitatively on four individual mouse liver RNA samples at each point. The *CYP3A11* mRNA level was normalized to the *GAPDH* mRNA level in the same samples. The *CYP3A11* mRNA level of the control was assigned as 100%. $^{\ddagger\ddagger}p < .01$ as compared with the control; $^{**}p < .01$ as compared with inducer-treated group. (B) Livers were excised and microsomes were isolated from livers 18 h after LPS treatment. ERND catalytic activity was measured as described under Materials and Methods. Data are expressed as means ± SEM of nine mice. $^{\S\S}p < .01$ as compared with the control; $^{**}p < .01$ as compared with inducer-treated group.

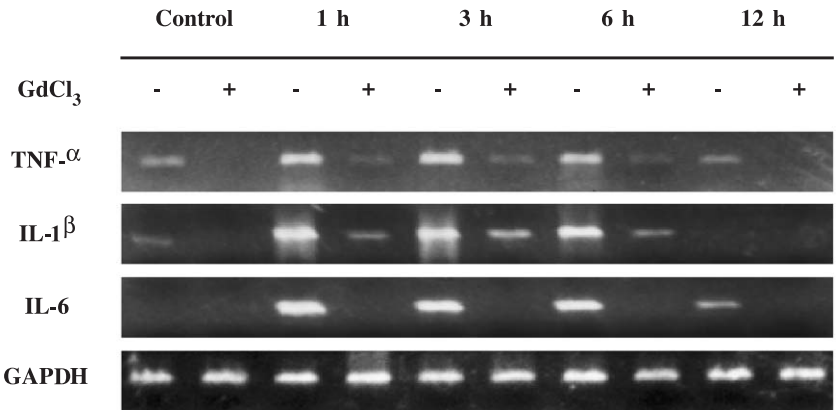

Fig. 3. Effects of $GdCl_3$ on LPS-induced *TNF-α*, *IL-1β*, and *IL-6* mRNA levels in mouse liver. Mice were administered a single dose of $GdCl_3$ (10 mg/kg, i.v.) 24 h before LPS (1 mg/kg, i.p.) treatment. Livers were excised and total RNA was extracted 1, 3, 6, and 12 h after LPS treatment. Total cellular RNA extracted from four mouse livers was pooled at each time point. *TNF-α*, *IL-1β*, and *IL-6* mRNA levels in mouse livers were determined using RT-PCR.

pellet was resuspended in Tris–chloride buffer, pH 7.4, containing 20% glycerol, with a ground-glass tissue grinder and stored at −80°C. Protein concentrations of microsome samples were measured according to the method of Lowry et al. [33], using bovine serum albumin as a standard.

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 [34] 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. Measurements of ERND catalytic activity were repeated twice for three separately prepared liver microsome samples.

Statistical analysis

The *PXR* and *CYP3A11* mRNA levels were normalized to the GAPDH mRNA level in the same samples. *PXR* and *CYP3A11* mRNA levels of the control was set at 100%. Quantified data from analysis of plasma nitrite and nitrate concentration, RT-PCR, and ERND assay were expressed as means ± SEM at each point. ANOVA and the Student–Newmann–Keuls post hoc test were used to determine differences between the treated animals and the control and statistical significance.

RESULTS

Effects of LPS on constitutive expressions of PXR and CYP3A11 mRNAs

The effects of LPS on the constitutive expression of *PXR* and *CYP3A11* mRNA are shown in Fig. 1. As

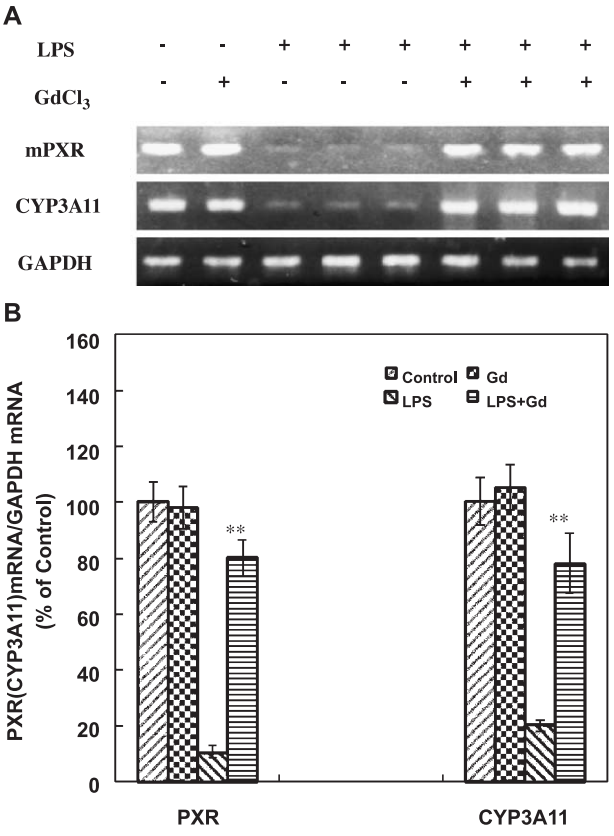

Fig. 4. Effects of $GdCl_3$ on LPS-induced downregulation of *PXR* and *CYP3A11* mRNA levels. Mice were administered a single dose of $GdCl_3$ (10 mg/kg, i.v.) 24 h before LPS (1.0 mg/kg, i.p.) treatment. (A) Livers were excised and total RNA was extracted 12 h after LPS treatment. Total RNA extracted from four mouse livers was pooled on each treatment. *PXR* and *CYP3A11* mRNA levels were determined using RT-PCR. (B) Quantitative analysis of *PXR* mRNA was performed on four individual mouse liver RNA samples at each point. *PXR* and *CYP3A11* mRNA levels were normalized to the *GAPDH* mRNA level in the same samples. The *PXR* and *CYP3A11* mRNA levels of the control were set at 100%. $^{**}p < .01$ as compared with LPS-treated group.

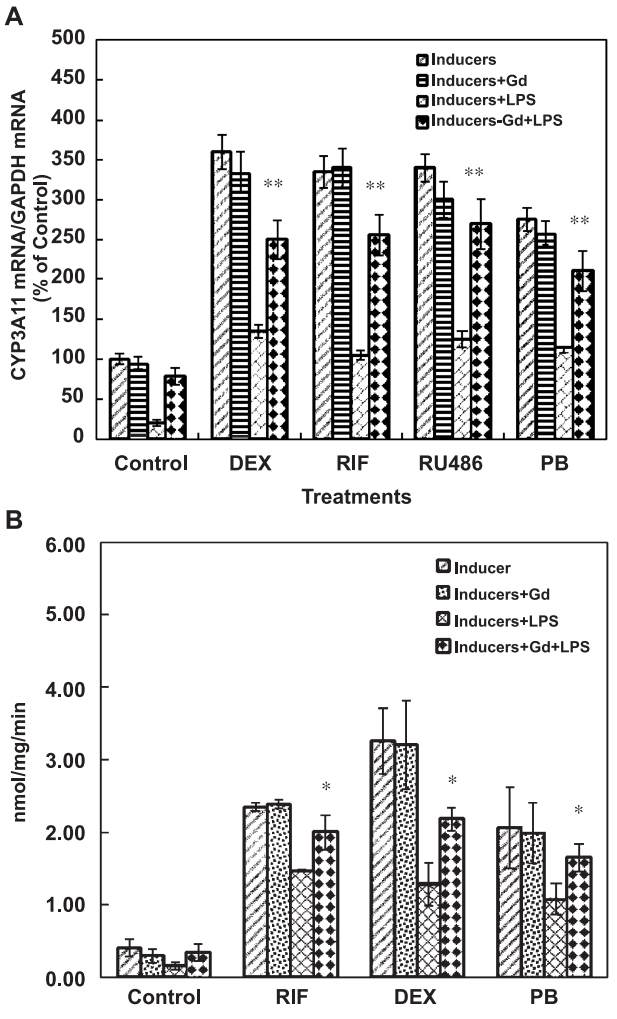

Fig. 5. Effects of $GdCl_3$ on LPS-induced downregulation of inducible *CYP3A11* mRNA levels and ERND catalytic activity. Mice were administered Dex (40 mg/kg, i.p.), RIF (50 mg/kg, i.g.), RU486 (50 mg/kg, s.c.), or PB (75mg/kg, i.p.) for 3 days. On the third day, mice were administered a single dose of $GdCl_3$ (10 mg/kg, i.v.). On the fourth day, mice were treated with LPS (1.0 mg/kg, i.p.) or saline. Two hours later, mice were administered Dex (40 mg/kg, i.p.), RIF (50 mg/kg,i.g.), RU486 (50 mg/kg, s.c.), or PB (75 mg/kg, i.p.). (A) Livers were excised and total RNA was extracted at 12 h after LPS treatment. *CYP3A11* mRNA levels were analyzed quantitatively on four individual mouse liver RNA samples at each point. The *CYP3A11* mRNA level was normalized to the *GAPDH* mRNA level in the same samples. The *CYP3A11* mRNA level of the control was set at 100%. $^{**}p < .01$ as compared with LPS/inducer-co-treated group. (B) Livers were excised and microsomes were isolated from livers 18 h after LPS treatment. ERND catalytic activity was measured as described under Materials and Methods. Data are expressed as means ± SEM of nine mice. $^{*}p < .05$ as compared with LPS/inducer-co-treated group.

expected, LPS significantly inhibited the constitutive expression of *PXR* mRNA. LPS downregulated *PXR* mRNA levels 6 h after LPS treatment, followed by suppression of *CYP3A11* mRNA in mouse liver. LPS-induced downregulation of *PXR* and *CYP3A11* mRNA levels lasted at least 24 h (Figs. 1A and 1B).

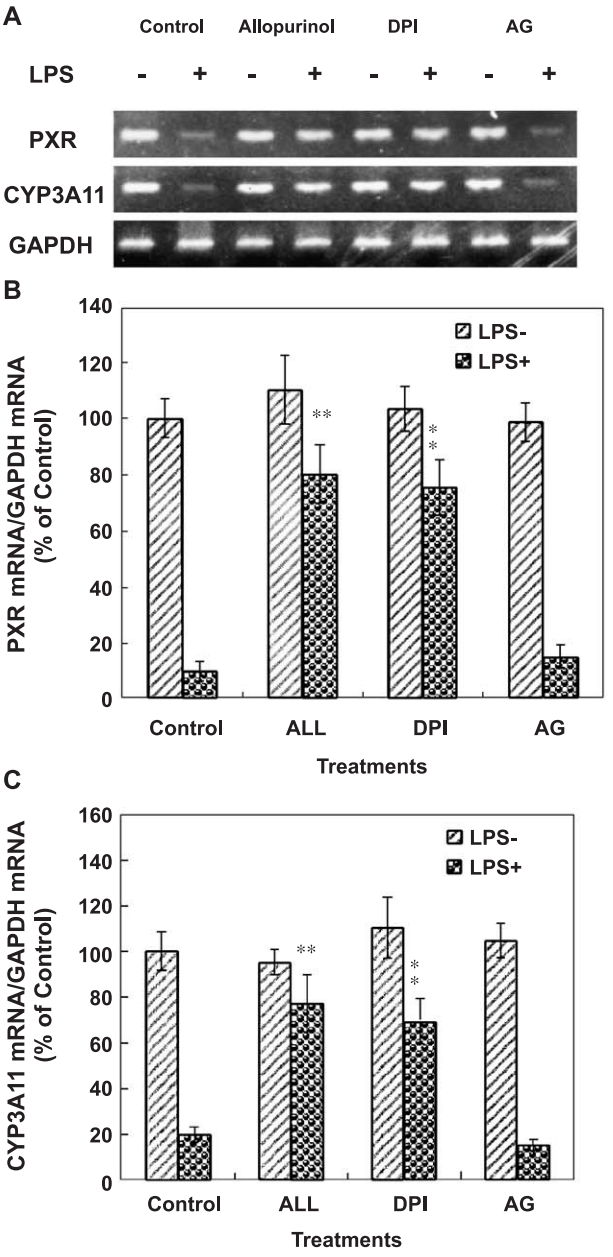

Fig. 6. Effects of ALL, DPI, and AG on LPS-induced downregulation of *PXR* and *CYP3A11* mRNA levels. Mice were administered allopurinol (100 mg/kg, i.g.), DPI (1.0 mg/kg, s.c.), or AG (150 mg/kg, i.p.) as described under Materials and Methods. (A) Livers were excised and total RNA was extracted 12 h after LPS treatment. Total RNA extracted from four mouse livers was pooled on each treatment. *PXR* and *CYP3A11* mRNA levels were determined using RT-PCR. (B) Quantitative analysis of *PXR* mRNA was performed on four individual mouse liver RNA samples at each point. The *PXR* mRNA level was normalized to the *GAPDH* mRNA level in the same samples. The *PXR* mRNA level of the control was set at 100%. $^{**}p < .01$ as compared with LPS-treated control group. (C) Quantitative analysis of *CYP3A11* mRNA was performed on four individual mouse liver RNA samples at each point. The *CYP3A11* mRNA level was normalized to the *GAPDH* mRNA level in the same samples. The *CYP3A11* mRNA level of the control was assigned as 100%. $^{**}p < .01$ as compared with LPS-treated control group.

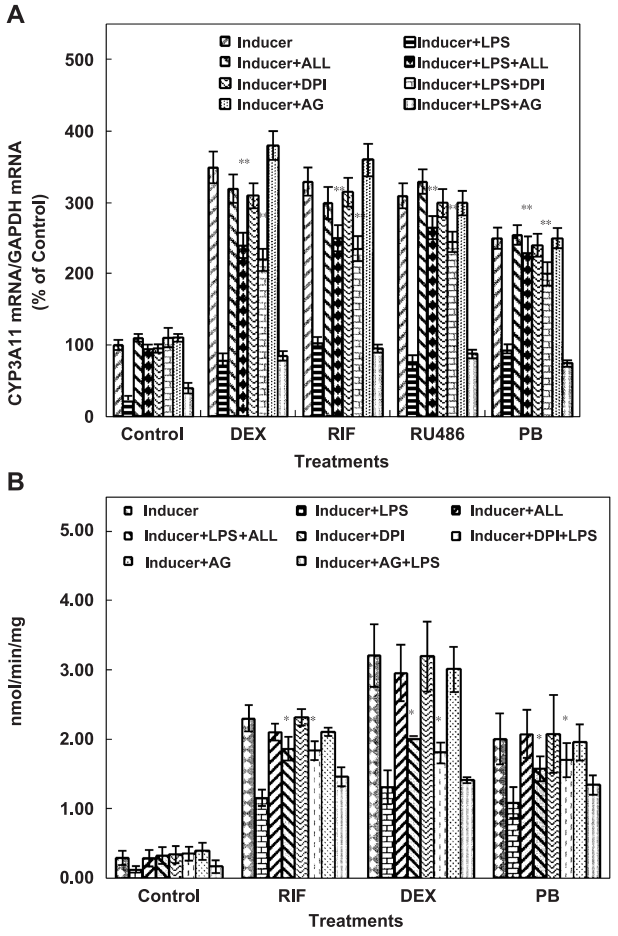

Fig. 7. Effects of ALL, DPI, and AG on LPS-induced downregulation of inducible *CYP3A11* mRNA levels and ERND catalytic activity. All groups except the control were administered Dex (40 mg/kg, i.p.), RIF (50 mg/kg, i.g.), RU486 (50 mg/kg, s.c.), or PB (75mg/kg, i.p.) for 3 days before LPS treatment. Mice were administered either ALL (100 mg/kg, i.g.), DPI (1.0 mg/kg, s.c.), or AG (150 mg/kg, i.p.) 48, 24, and 2 h before LPS treatment. On the fourth day, mice were injected with LPS (1.0 mg/kg, i.p.) and *CYP3A* inducers, followed by either ALL (100 mg/kg, i.g.), DPI (1.0 mg/kg, s.c.), or AG (150 mg/kg, i.p.) 2 and 6 h after LPS treatment. (A) Livers were excised and total RNA was extracted 12 h after LPS treatment. *CYP3A11* mRNA levels were analyzed quantitatively on four individual mouse liver RNA samples at each point. The *CYP3A11* mRNA level was normalized to the *GAPDH* mRNA level in the same samples. The *CYP3A11* mRNA level of the control was assigned as 100%. $^{**}p < .01$ as compared with LPS/inducer-co-treated group. (B) Livers were excised and microsomes were isolated from livers 18 h after LPS treatments. ERND catalytic activity was measured as described under Materials and Methods. Data are expressed as means ± SEM of nine mice. $^{*}p < .05$ as compared with LPS/inducer-co-treated groups.

To explore whether a dose–response relationship existed, mice were injected with different doses of LPS (0.1–5.0 mg/kg, i.p.). *PXR* and *CYP3A11* mRNA levels in liver were determined 12 h after LPS treatment. Results showed that LPS significantly downregulates *PXR* and *CYP3A11* mRNA levels in a dose-respondent manner (Figs. 1C and 1D).

Effects of LPS on inducible CYP3A11 mRNA and ERND catalytic activities

The effects of LPS on the inducible expression of *CYP3A11* mRNA are shown in Fig. 2A. Results indicate that Dex, RIF, RU486, or PB alone caused a 2.5- to 4.0-fold induction of *CYP3A11* mRNA levels. LPS repressed the upregulation of *CYP3A11* mRNA levels in mice pretreated with the PXR ligands Dex, RIF, RU486, and PB.

ERND was used as an indicator of CYP3A catalytic activity in this study. As shown in Fig. 2B, RU486 induced about a 2-fold increase in ERND activity. Dex, RIF, or PB alone caused a 5- to10-fold induction of ERND catalytic activity. Such an induction, however, was significantly reduced by the administration of LPS.

Effects of $GdCl_3$ on TNF-α, IL-1β, IL-6, PXR, and CYP3A11 mRNA expression and ERND catalytic activity

As further verification of the efficacy of $GdCl_3$, proinflammatory cytokine mRNA levels for *TNF-α*, *IL-1β*, and *IL-6* in liver of LPS-treated and LPS/ $GdCl_3$-co-treated mice were determined 1, 3, 6, and 12 h after LPS treatment. As shown in Fig. 3, mRNA levels for *TNF-α*, *IL-1β*, and *IL-6* significantly increased in livers of LPS-treated animals. $GdCl_3$ pretreatment blocked LPS-mediated *TNF-α*, *IL-1β*, and *IL-6* mRNA expression in mouse livers.

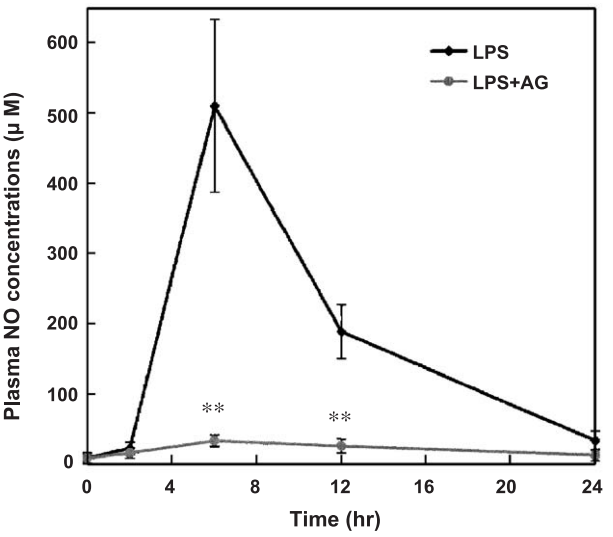

Fig. 8. Effects of AG on LPS-evoked plasma NO levels. Mice were administered AG (150 mg/kg, i.p.) 48, 24, and 2 h before LPS administration. Mice were then injected with LPS (1.0 mg/kg, i.p.), followed by repeated doses of AG at 4-hr intervals. Plasma concentrations of nitrite plus nitrate were analyzed via the Griess reaction, as described under Materials and Methods. Data are expressed as means ± SEM of six mice at each point. $^{*}p < .01$ versus LPS-treated control.

The effects of $GdCl_3$ on LPS-induced downregulation of constitutive *PXR* and *CYP3A11* mRNA levels were analyzed. As shown in Fig. 4, $GdCl_3$ pretreatment significantly attenuated LPS-induced downregulation of *PXR* and *CYP3A11* mRNA levels. Effects of $GdCl_3$ on LPS-induced downregulation of inducible *CYP3A11* mRNA levels and ERND catalytic activity were then evaluated. As shown in Fig. 5A, $GdCl_3$ significantly attenuated LPS-induced downregulation of Dex-, RIF-, RU486- and PB-inducible *CYP3A11* mRNA in mouse liver. $GdCl_3$ also significantly attenuated LPS-induced downregulation of Dex-, RIF-, RU486-, and PB-inducible ERND catalytic activity in mouse liver (Fig. 5B).

Effects of ALL and DPI on LPS-induced downregulation of PXR and CYP3A11 mRNA expression and ERND catalytic activity

The effects of ALL and DPI on LPS-induced downregulation of constitutive *PXR* and *CYP3A11* mRNA levels are shown in Fig. 6. Results indicate that LPS-induced downregulation of constitutive *PXR* and *CYP3A11* mRNAs was significantly attenuated in mice pretreated with either ALL or DPI.

The effects of ALL and DPI on LPS-induced downregulation of inducible *CYP3A11* mRNA levels and ERND catalytic activity were analyzed. As shown in Fig. 7A, ALL and DPI pretreatments significantly attenuated LPS-induced downregulation of Dex-, RIF-, RU486-, and PB-inducible *CYP3A11* mRNA levels. In addition, ALL and DPI pretreatments also attenuated LPS-induced downregulation of ERND catalytic activity in mouse liver (Fig. 7B).

Effects of AG on plasma NO levels, PXR and CYP3A11 mRNA expression and ERND catalytic activity

As verification of the efficacy of AG, plasma NO levels in LPS-treated and LPS/AG-co-treated mice were determined 2, 6, 12 and 24 h after LPS treatment. As expected, plasma NO levels significantly increased 6 and 12 h after LPS treatment. AG inhibited the LPS-evoked increase in NO levels (Fig. 8).

The effects of AG on LPS-induced downregulation of constitutive *PXR* and *CYP3A11* mRNA levels are illustrated in Fig. 6. Results show that AG has no effects on LPS-induced decreases in *PXR* and *CYP3A11* mRNA levels. The effects of AG on LPS-induced downregulation of inducible *CYP3A11* mRNA levels and ERND catalytic activity are illustrated in

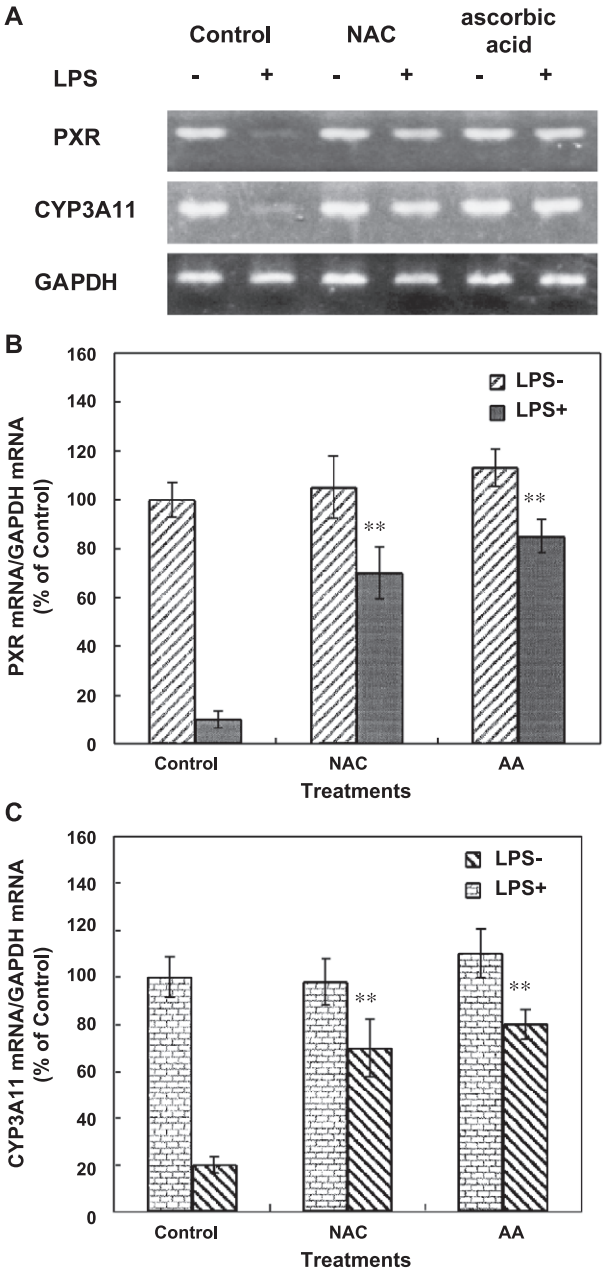

Fig. 9. Effects of NAC and AA on LPS-induced downregulation of *PXR* and *CYP3A11* mRNA levels. Mice were injected with either NAC (150 mg/kg, i.v.) or AA (400 mg/kg, i.v.) 48, 24, and 2 h before LPS treatment. Mice were then injected with LPS (1.0 mg/kg, i.p.), followed by either NAC (150 mg/kg, i.v.) or AA (400 mg/kg, i.v.) 2 and 6 h after LPS treatment. (A) Livers were excised and total RNA was extracted 12 h after LPS treatment. Total RNA extracted from four mouse livers was pooled on each treatment. *PXR* and *CYP3A11* mRNA levels were determined using RT-PCR. (B) Quantitative analysis of *PXR* mRNA was performed on four individual mouse liver RNA samples at each point. The *PXR* mRNA level was normalized to the *GAPDH* mRNA level in the same samples. The *PXR* mRNA level of the control was assigned as 100%. $^{**}p < .01$ as compared with LPS-treated control group. (C) Quantitative analysis of *CYP3A11* mRNA was performed on four individual mouse liver RNA samples at each point. The *CYP3A11* mRNA level was normalized to the *GAPDH* mRNA level in the same samples. The *CYP3A11* mRNA level of the control was set at 100%. $^{**}p < .01$ as compared with the LPS-treated control group.

Figs. 7A and 7B. Results show that AG has no effect on LPS-evoked downregulation of Dex-, RIF-, RU486-, and PB-inducible *CYP3A11* mRNA levels and ERND catalytic activity in mouse liver.

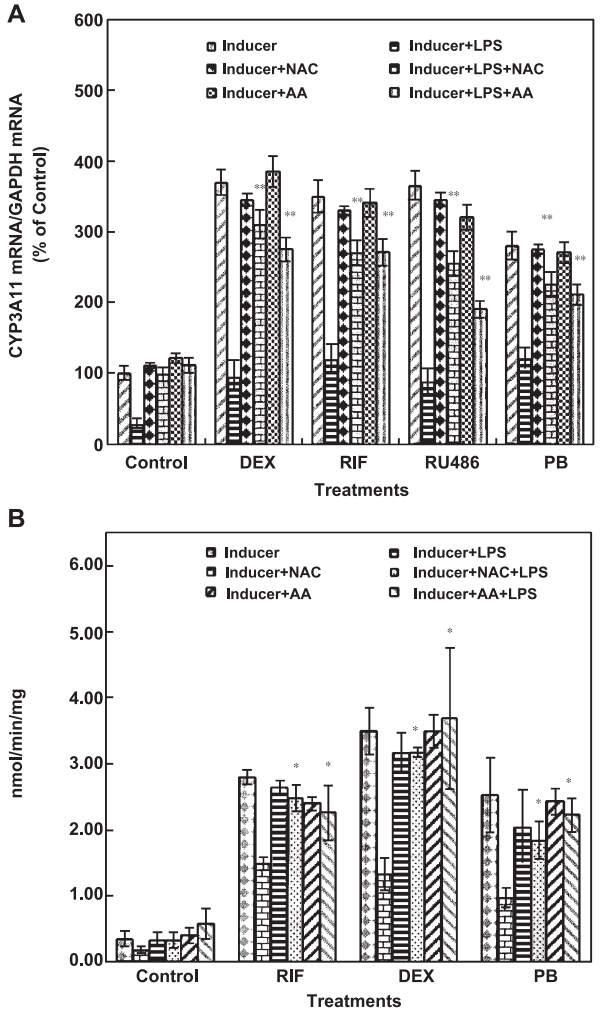

Fig. 10. Effects of NAC and AA on LPS-induced downregulation of inducible *CYP3A11* mRNA and ERND catalytic activity. Mice were administered Dex (40 mg/kg, i.p.), RIF (50 mg/kg, i.g.), RU486 (50 mg/kg, s.c.), or PB (75mg/kg, i.p.) for 3 days. Mice were pretreated with NAC (150 mg/kg, i.v.), or AA (400 mg/kg, i.v.) 48, 24, and 2 h before LPS administration. On the fourth day, mice were administered LPS (1.0 mg/kg, i.p.) and CYP3A inducers, followed by either NAC (150 mg/kg, i.v.) or AA (400 mg/kg, i.v.) 2 and 6 h after LPS treatment. (A) Livers were excised and total RNA was extracted 12 h after LPS treatment. *CYP3A11* mRNA levels of four individual mouse liver RNA samples at each point were analyzed quantitatively. The *CYP3A11* mRNA level was normalized to the *GAPDH* mRNA level in the same samples. The *CYP3A11* mRNA level of the control was set at 100%. $^{**}p < .01$ as compared with the LPS/inducer-co-treated group. (B) Livers were excised and microsomes were isolated from livers 18 h after LPS treatment. ERND catalytic activity was measured as described under Materials and Methods. Data are expressed as means ± SEM of nine mice. $^{*}p < .05$ as compared with LPS/inducer-co-treated groups.

Effects of NAC and AA on LPS-induced downregulation of PXR and CYP3A11 mRNA levels and ERND catalytic activity

The effects of two antioxidants, NAC and AA, on LPS-induced downregulation of *PXR* and *CYP3A11* mRNA levels and ERND catalytic activity were analyzed. As shown in Fig. 9, LPS-induced downregulation of constitutive *PXR* and *CYP3A11* mRNA was significantly attenuated in mice pretreated with either NAC or AA. These antioxidants also prevented LPS-evoked downregulation of Dex-, RIF-, RU486-, and PB-inducible *CYP3A11* mRNA expression (Fig. 10A) and ERND catalytic activity (Fig. 10B) in mouse liver.

DISCUSSION

Pregnane X receptor is a member of the nuclear receptor superfamily that regulates target gene transcription in a ligand-dependent manner. The in vivo effects of LPS on expressions of *PXR* and its target gene *CYP3A* in mouse liver were investigated in this study. Results indicate that LPS significantly inhibits the constitutive expression of *PXR* mRNA in a dose-respondent manner, followed by suppression of *CYP3A11* mRNA and ERND catalytic activity in mouse liver. LPS also reverses the upregulation of *CYP3A11* mRNA levels in mice pretreated with PXR ligands Dex, RIF, RU486, and PB. These results are in agreement with earlier work by Beigneux et al. [13]. It has been demonstrated that mouse liver CYP3A is the major enzyme catalyzing erythromycin N-demethylation [37]. Thus, ERND was used as an indicator of CYP3A catalytic activity in this study. The present results indicate that LPS significantly represses the upregulation of ERND catalytic activity in mouse liver microsomes.

Numerous studies have demonstrated that pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, are involved in LPS-induced downregulation of cytochromes P450 [35,36]. Kupffer cells, the resident macrophages of the liver, are the main sources of pro-inflammatory cytokines and produce TNF-α, IL-1β, and IL-6 after LPS challenge [38,39]. Therefore, Kupffer cells might play an important role in LPS-induced downregulation of cytochromes P450 in liver. In accordance with this view, an in vitro study by Milosevic et al. [40], comparing the responses of hepatocytes co-cultured with Kupffer cells with those of hepatocytes alone, showed that LPS-induced suppression of PB-inducible *CYP2B1* mRNA was mediated by TNF-α released from the Kupffer cells. It has been demonstrated that $GdCl_3$ pretreatment has an effect on preventing LPS-evoked release of O^{2-}, NO, and pro-inflammatory cytokines from Kupffer cells [41–44].

In the present study, to determine the in vivo role of Kupffer cells in LPS-induced downregulation of *CYP3A* expression, mice were treated with $GdCl_3$, a selective Kupffer cell toxicant, to inactivate Kupffer cells. Results indicate that $GdCl_3$ pretreatment almost blocks LPS-mediated *TNF-α*, *IL-1β*, and *IL-6* mRNA expression in mouse livers. Interestingly, LPS-induced downregulation of *PXR* and *CYP3A11* mRNA levels in liver is significantly attenuated in mice pretreated with $GdCl_3$. A single dose of $GdCl_3$ (10 mg/kg) also significantly attenuates the repressive effects of LPS on Dex-, RIF-, RU486-, and PB-inducible *CYP3A11* mRNA expression and ERND activity in mouse liver. These results demonstrate in vivo the role of Kupffer cells in LPS-induced downregulation of nuclear receptor *PXR* and its target gene *CYP3A* in mouse liver.

Reactive oxygen species are known to influence the expression of a number of genes and signal transduction pathways [45,46]. Kupffer cells are the main sources of ROS in liver. LPS, a potent activator of macrophages, stimulates Kupffer cells to generate ROS, such as O^{2-} and NO [47,48]. Elevated levels of O^{2-} and NO in Kupffer cells are responsible for activation of nuclear factor-kB and release of pro-inflammatory cytokines [49,50]. Therefore, the relationship between ROS, especially Kupffer cell-derived ROS, and LPS-induced downregulation of nuclear receptor PXR and its target gene *CYP3A* is especially interesting. Two of the important sources of ROS in Kupffer cells are xanthine oxidase and NADPH oxidase [51–53]. To determine the role of ROS on LPS-induced downregulation of *PXR* and *CYP3A*, ALL, an inhibitor of xanthine oxidase and an antioxidant, and DPI, an inhibitor of NADPH oxidase, were used to inhibited LPS-induced ROS production. Interestingly, LPS-induced downregulation of *PXR* and *CYP3A11* mRNA was significantly attenuated in mice pretreated with either DPI or ALL. Either DPI or allopurinol pretreatment also partially attenuated LPS-induced downregulation of Dex-, RIF-, RU486-, and PB-inducible *CYP3A11* mRNA levels and ERND catalytic activity in mouse liver. Moreover, NAC and AA, two well-known antioxidants, significantly attenuated LPS-induced downregulation of *PXR* and *CYP3A11* mRNA levels. Either of these antioxidants also prevented the repressive effects of LPS on Dex-, RIF-, RU486-, and PB-inducible *CYP3A11* mRNA and ERND catalytic activity in mouse liver. These results suggest that ROS are involved in LPS-induced downregulation of nuclear receptor *PXR* and its target gene *CYP3A* in mouse liver.

The role of nitric oxide (NO) in LPS-evoked downregulation of *PXR* and *CYP3A* was also investigated. AG, a selective inhibitor of inducible nitric oxide synthase (iNOS), was used to inhibit the release of NO in LPS-treated mice. Interestingly, this iNOS inhibitor has no effect on LPS-induced downregulation of *PXR* and *CYP3A11* mRNA, which is in agreement with the earlier work by Sewer et al. [11], suggesting that LPS-induced downregulation of nuclear receptor *PXR* and its target gene *CYP3A* in mouse liver is independent of NO production.

As noted above, however, $GdCl_3$ did attenuate but did not completely block LPS-induced downregulation of *PXR* and *CYP3A*, suggesting that Kupffer cells only partially contribute to LPS-induced downregulation of PXR and *CYP3A*. Indeed, NADPH oxidase is also present in hepatocytes. Recent studies found that hepatocytes express CD_{14} and TLR-4 [54,55]. CD_{14} mRNA and protein levels on hepatocytes increase rapidly during endotoxemia [56]. Therefore, our results do not exclude the involvement of other mechanisms. Additional work is required to determine whether LPS can act directly on hepatocytes to downregulate *PXR* and its target gene *CYP3A*.

In summary, the present results allow us to reach the following conclusions. First, LPS significantly inhibits the expression of *PXR* and its target gene *CYP3A* in mouse liver. Second, Kupffer cells partially contribute to LPS-induced downregulation of PXR and *CYP3A* in mouse liver. And third, LPS-induced downregulation of *PXR* and *CYP3A* in mouse liver is mediated, at least in part, by ROS.

Acknowledgments—The project (30371667) was supported by the National Natural Science Foundation of China. We gratefully acknowledge Professor Yuan Wang and Dr. Qing-Lin Fan for advice on molecular biology and techniques.

REFERENCES

[1] Thummel, K. E.; Wilkinson, G. R. In vitro and in vivo drug interactions involving human CYP3A. *Annu. Rev. Pharmacol. Toxicol.* **38:**389–430; 1998.

[2] Quattrochi, L. C.; Guzelian, P. S. CYP3A regulation: from pharmacology to nuclear receptors. *Drug Metab. Dispos.* **29:** 615–622; 2001.

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

[4] Kliewer, S. A.; Moore, J. T.; Wade, L.; Staudinger, J. L.; Watson, M. A.; Jones, S. A.; McKee, D. D.; Oliver, B. B.; Willson, T. M.; Zetterstrom, R. H.; Perlmann, T.; Lehmann, J. M. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* **92:**73–82; 1998.

[5] Lehmann, J. M.; McKee, D. D.; Watson, M. A.; Willson, T. M.; Moore, J. T.; Kliewer, S. A. The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J. Clin. Invest.* **102:** 1016–1023; 1998.

[6] Bertilsson, G.; Heidrich, J.; Svensson, K.; Asman, M.; Jendeberg, L.; Sydow-Backman, M.; Ohlsson, R.; Postlind, H.; Blomquist, P.; Berkenstam, A. Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc. Natl. Acad. Sci. USA* **95:**12208–12213; 1998.

[7] Moore, L. B.; Parks, D. J.; Jones, S. A.; Bledsoe, R. K.; Consler, T. G.; Stimmel, J. B.; Goodwin, B.; Liddle, C.; Blanchard, S. G.; Willson, T. M.; Collins, J. L.; Kliewer, S. A. Orphan nuclear

receptors constitutive androstane receptor and pregnane X receptor share xenobiotic and steroid ligands. *J. Biol. Chem.* **275:** 15122–15127; 2000.

[8] Xie, W.; Barwick, J. L.; Simon, C. M.; Pierce, A. M.; Safe, S.; Blumberg, B.; Guzelian, P. S.; Evans, R. M. Reciprocal activation of xenobiotic response genes by nuclear receptors SXR/PXR and CAR. *Genes Dev.* **14:**3014–3023; 2000.

[9] Morgan, E. T. Regulation of cytochrome p450 by inflammatory mediators: why and how? *Drug Metab. Dispos.* **29:**207–212; 2001.

[10] Morgan, E. T. Regulation of cytochromes P450 during inflammation and infection. *Drug Metab. Rev.* **29:**1129–1188; 1997.

[11] Sewer, M. B.; Barclay, T. B.; Morgan, E. T. Downregulation of cytochrome P450 mRNAs and proteins in mice lacking a functional NOS2 gene. *Mol. Pharmacol.* **54:**273–279; 1998.

[12] Li-Masters, T.; Morgan, E. T. Effects of bacterial lipopolysaccharide on phenobarbital-induced CYP2B expression in mice. *Drug Metab. Dispos.* **29:**252–257; 2001.

[13] Beigneux, A. P.; Moser, A. H.; Shigenaga, J. K.; Grunfeld, C.; Feingold, K. R. 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; 2002.

[14] Sachdeva, K.; Yan, B.; Chichester, C. O. 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; 2003.

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

[16] Nadin, L.; Butler, A. M.; Farrell, G. C.; Murray, M. Pretranslational downregulation of cytochromes P450 2C11 and 3A2 in male rat liver by tumor necrosis factor alpha. *Gastroenterology* **109:**198–205; 1995.

[17] Jover, R.; Bort, R.; Gomez-Lechon, M. J.; Castell, J. V. Downregulation of human CYP3A4 by the inflammatory signal interleukin-6: molecular mechanism and transcription factors involved. *FASEB. J.* **16:**1799–1801; 2002.

[18] Tran-Thi, T. A.; Decker, K.; Baeuerle, P. A. Differential activation of transcription factors NF-kappa B and AP-1 in rat liver macrophages. *Hepatology* **22:**613–619; 1995.

[19] Warren, G. W.; Poloyac, S. M.; Gary, D. S.; Mattson, M. P.; Blouin, R. A. 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; 1999.

[20] Siewert, E.; Bort, R.; Kluge, R.; Heinrich, P. C.; Castell, J.; Jover, R. Hepatic cytochrome P450 downregulation during aseptic inflammation in the mouse is interleukin 6 dependent. *Hepatology* **32:**49–55; 2000.

[21] Yee, S. B.; Ganey, P. E.; Roth, R. A. The role of Kupffer cells and TNF-α in monocrotaline and bacterial lipopolysaccharide-induced liver injury. *Toxicol. Sci.* **71:**124–132; 2002.

[22] Kono, H.; Rusin, I.; Bradford, B. U.; Corror, H. D.; Mason, R. P.; Thurman, R. G. Allopurinol prevents early alcohol-induced liver injury in rats. *J. Pharmacol. Exp. Ther.* **293:**296–303; 2000.

[23] Kono, H.; Rusin, I.; Uesugi, T.; Yamashina, S.; Corror, H. D.; Dikalova, A.; Mason, R. P.; Thurman, R. G. Diphenyleneiodonium sulfate, an NADPH oxidase inhibitor, prevents early alcohol-induced liver injury in the rat. *Am. J. Physiol.* **280:**G1005–G1012; 2001.

[24] Sewer, M. B.; Morgan, E. T. Downregulation of the expression of three major rat liver cytochrome P450s by endotoxin in vivo occurs independently of nitric oxide production. *J. Pharmacol. Exp. Ther.* **287:**352–358; 1998.

[25] Victor, V. M.; Rocha, M.; De la Fuente, M. *N*-Acetylcysteine protects mice from lethal endotoxemia by regulating the redox state of immune cells. *Free Radic. Res.* **37:**919–929; 2003.

[26] Tracey, W. R.; Tse, J.; Carter, G. Lipopolysaccharide-induced changes in plasma nitrite and nitrate concentrations in rats and mice: pharmacological evaluation of nitric oxide synthase inhibitors. *J. Pharmacol. Exp. Ther.* **272:**1011–1015; 1995.

[27] Grisham, M. B.; Johnson, G. G.; Lancaster, J. R., Jr. Quantitation of nitrate and nitrite in extracellular fluids. *Methods Enzymol.* **268:**237–246; 1996.

[28] Ueda, A.; Hamadeh, H. K.; Webb, H. K.; Yamamoto, Y.; Sueyoshi, T.; Afshari, C. A.; Lehmann, J. M.; Negishi, M. Diverse roles of the nuclear orphan receptor car in regulating hepatic genes in response to phenobarbital. *Mol. Pharmacol.* **61:**1–6; 2002.

[29] Li, J.; Ning, G.; Duncan, S. A. Mammalian hepatocyte differentiation requires the transcription factor HNF-4alpha. *Genes Dev.* **14:**464–474; 2000.

[30] Ehlers, S. M.; Mielke, E. A.; Blankenstein, T.; Hahn, H. Kinetic analysis of cytokine gene expression in the livers of naïve and immune mice infected with *Listeria monocytogenes*. *J. Immunol.* **149:**3016–3022; 1992.

[31] Murray, L. J.; Lee, R.; Martens, C. In vivo cytokine gene expression in T cell subsets of the autoimmune MRL/Mp-lpr/lpr mouse. *Eur. J. Immunol.* **20:**163–170.

[32] Haugen, D. A.; Coon, M. J. Properties of electrophoretically homogeneous phenobarbital-inducible and beta-naphthoflavone-inducible forms of liver microsomal cytochrome P-450. *J. Biol. Chem.* **251:**7929–7939; 1976.

[33] Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:**265–275; 1951.

[34] Werringloer, J. Assay of fomaldehyde generated during microsomal oxidation reactions. *Methods Enzymol.* **52:**297–302; 1978.

[35] Sewer, M. B.; Morgan, E. T. Nitric oxide-independent suppression of P450 2C11 expression by interleukin-1beta and endotoxin in primary rat hepatocytes. *Biochem. Pharmacol.* **54:**729–737; 1997.

[36] Monshouwer, M.; McLellan, R. A.; Delaporte, E.; Witkamp, R. F.; van Miert, A. S.; Renton, K. W. Differential effect of pentoxifylline on lipopolysaccharide-induced downregulation of cytochrome P450. *Biochem. Pharmacol.* **52:**1195–1200; 1996.

[37] Lan, L. B.; Dalton, J. T.; Schuetz, E. G. Mdr1 limits CYP3A metabolism in vivo. *Mol. Pharmacol.* **58:**863–869; 2000.

[38] Aono, K.; Isobe, K.; Kiuchi, K.; Fan, Z. H.; Ito, M.; Takeuchi, A.; Miyachi, M.; Nakashima, I.; Nimura, Y. In vitro and in vivo expression of inducible nitric oxide synthase during experimental endotoxemia: involvement of other cytokines. *J. Cell Biochem.* **65:**349–358; 1997.

[39] Luster, M. I.; Germolec, D. R.; Yoshida, T.; Kayama, F.; Thompson, M. Endotoxin-induced cytokine gene expression and excretion in the liver. *Hepatology* **19:**480–488; 1994.

[40] Milosevic, N.; Schawalder, H.; Maier, P. Kupffer cell-mediated differential downregulation of cytochrome P450 metabolism in rat hepatocytes. *Eur. J. Pharmacol.* **368:**75–87; 1999.

[41] Kono, H.; Fujii, H.; Matsuda, M.; Yamamoto, M.; Matsumoto, Y. Gadolinium chloride prevents mortality in hepatectomized rats given endotoxin. *J. Surg. Res.* **96:**204–210; 2001.

[42] Roland, C. R.; Naziruddin, B.; Mohanakumar, T.; Flye, M. W. Gadolinium chloride inhibits Kupffer cell nitric oxide synthase (iNOS) induction. *J. Leukoc. Biol.* **60:**487–492; 1996.

[43] Rizzardini, M.; Zappone, M.; Villa, P.; Gnocchi, P.; Sironi, M.; Diomede, L.; Meazza, C.; Monshouwer, M.; Cantoni, L. Kupffer cell depletion partially prevents hepatic heme oxygenase 1 messenger RNA accumulation in systemic inflammation in mice: role of interleukin 1beta. *Hepatology* **27:**703–710; 1998.

[44] Liu, P.; McGuire, G. M.; Fisher, M. A.; Farhood, A.; Smith, C. W.; Jaeschke, H. Activation of Kupffer cells and neutrophils for reactive oxygen formation is responsible for endotoxin-enhanced liver injury after hepatic ischemia. *Shock* **3:**56–62; 1995.

[45] Allen, R. G. Oxidative stress and superoxide dismutase in development, aging and gene regulation. *Age* **21:**47–76; 1998.

[46] Allen, R. G.; Tresini, M. Oxidative stress and gene regulation. *Free Radic. Biol. Med.* **28:**463–499; 2000.

[47] Bautista, A. P.; Meszaros, K.; Bojta, J.; Spitzer, J. J. Superoxide anion generation in the liver during the early stage of endotoxemia in rats. *J. Leukoc. Biol.* **48:**123–128; 1990.

[48] Hida, A. I.; Kawabata, T.; Minamiyama, Y.; Mizote, A.; Okada, S. Saccharated colloidal iron enhances lipopolysaccharide-induced nitric oxide production in vivo. *Free Radic. Biol. Med.* **34:** 1426–1434; 2003.
[49] Wheeler, M. D.; Yamashina, S.; Froh, M.; Rusyn, I.; Thurman, R. G. Adenoviral gene delivery can inactivate Kupffer cells: role of oxidants in NF-kappaB activation and cytokine production. *J. Leukoc. Biol.* **69:**622–630; 2001.
[50] Sass, G.; Koerber, K.; Bang, R.; Guehring, H.; Tiegs, G. Inducible nitric oxide synthase is critical for immune-mediated liver injury in mice. *J. Clin. Invest.* **107:**439–447; 2001.
[51] Wiezorek, J. S.; Brown, D. H.; Kupperman, D. E.; Brass, C. A. Rapid conversion to high xanthine oxidase activity in viable Kupffer cells during hypoxia. *J. Clin. Invest.* **94:**2224–2230; 1994.
[52] Potoka, D. A.; Takao, S.; Owaki, T.; Bulkley, G. B.; Klein, A. S. Endothelial cells potentiate oxidant-mediated Kupffer cell phagocytic killing. *Free Radic. Biol. Med.* **24:**1217–1227; 1998.
[53] Kono, H.; Rusyn, I.; Yin, M.; Gabele, E.; Yamashina, S.; Dikalova, A.; Kadiiska, M. B.; Connor, H. D.; Mason, R. P.; Segal, B. H.; Bradford, B. U.; Holland, S. M.; Thurman, R. G. NADPH oxidase-derived free radicals are key oxidants in alcohol-induced liver disease. *J. Clin. Invest.* **106:**867–872; 2000.
[54] Su, G. L.; Dorko, K.; Strom, S. C.; Nussler, A. K.; Wang, S. C. CD14 expression and production by human hepatocytes. *J. Hepatol.* **31:**435–442; 1999.
[55] Liu, S.; Gallo, D. J.; Green, A. M.; Williams, D. L.; Gong, X.; Shapiro, R. A.; Gambotto, A. A.; Humphris, E. L.; Vodovotz, Y.; Billiar, T. R. Role of toll-like receptors in changes in gene expression and NF-kappa B activation in mouse hepatocytes stimulated with lipopolysaccharide. *Infect. Immun.* **70:**3433–3442; 2002.
[56] Liu, S.; Khemlani, L. S.; Shapiro, R. A.; Johnson, M. L.; Liu, K.; Geller, D. A.; Watkins, S. C.; Goyert, S. M.; Billiar, T. R. Expression of CD14 by hepatocytes: upregulation by cytokines during endotoxemia. *Infect. Immun.* **66:**5089–5098; 1998.

ABBREVIATIONS

AA — ascorbic acid
AG — aminoguanidine
ALL — allopurinol
cDNA — complementary DNA
CYP3A — cytochrome P450 3A
Dex — dexamethasone
DPI — diphenyleneiodonium chloride
ERND — erythromycin *N*-demethylase
GAPDH — glyceraldehyde-3-phosphate dehydrogenase
$GdCl_3$ — gadolinium chloride
IFN — interferon
IL — interleukin
iNOS — inducible nitric oxide synthase
LPS — Lipopolysaccharide
NAC — *N*-acetylcysteine
NF-κB — nuclear factor κB
PB — phenobarbital
PXR — pregnane X receptor
RIF — rifampicin
ROS — reactive oxygen species
RT-PCR — reverse transcription polymerase chain reaction
RU486 — mifepristone
TNF-α — tumor necrosis factor α