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Maternally administered lipopolysaccharide (LPS) upregulates the expression of heme oxygenase-1 in fetal liver: The role of reactive oxygen species

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Abstract

Heme oxygenase-1 (HO-1) is an inducible enzyme that catalyzes the rate-limiting step in the degradation of heme to biliverdin, carbon monoxide and iron. Previous studies have demonstrated that lipopolysaccharide (LPS) upregulates the expression of HO-1 in adult mouse liver. The present study aimed to investigate the effects of maternal LPS exposure on the expression of HO-1 in fetal liver. The pregnant mice were intraperitoneally injected with different doses of LPS (1, 10, 75 μg/kg) on gestational day 17. Results showed that the expression of HO-1 in fetal liver was increased, beginning 2 h after LPS, being at the highest level 24 h after LPS, and remaining elevated up to 48 h after LPS, whereas HO-2, the constitutive form, did not change at the various time points observed. LPS-induced upregulation of HO-1 was blocked by alpha-phenyl-*N*-*t*-butylnitrone (PBN), a free radical spin trapping agent. Correspondingly, PBN pretreatment significantly attenuated LPS-induced lipid peroxidation and glutathione (GSH) depletion in fetal liver. However, aminoguanidine (AG), a selective inhibitor of inducible nitric oxide synthase (iNOS), and pentoxifylline (PTX), an inhibitor of tumor necrosis factor alpha (TNF-α) synthesis, had no effect on LPS-induced upregulation of HO-1 in fetal liver. In conclusion, reactive oxygen species (ROS), rather than TNF-α or nitric oxide (NO), are involved in LPS-induced upregulation of HO-1 in fetal liver. These results provide new evidence that maternal LPS exposure results in oxidative stress in fetuses, which may contribute to LPS-induced developmental toxicity.

Keywords: Lipopolysaccharide; Fetal liver; Heme oxygenase; Reactive oxygen species

1. Introduction

Heme oxygenase (HO) catalyzes the rate-limiting step in the degradation of heme to yield equimolar amounts of biliverdin, carbon monoxide, and iron. In mammals, biliverdin is rapidly converted to bilirubin by the enzyme biliverdin reductase. HO is involved in the control of vascular tone, regulating anti-inflammatory and anti-apoptotic responses as well as reducing oxidative stress and subsequent tissue damage in several organ systems (Morse and Choi, 2002; Wagener et al., 2003; Morita, 2005; Wu and Wang, 2005; Ryter et al., 2006). Three isoforms of the HO protein have been identified. HO-1 is a 32-kDa inducible form which is ubiquitously distributed in mammalian tissues but is expressed in high concentrations in the spleen and liver, areas of

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high erythrocyte turnover. A variety of stimuli have been shown to induce HO-1 expression, including oxidant stress, metalloporphyrins, transition metals, ischemia-reperfusion, cytokines, and hemin (Maines et al., 1993; Shibahara et al., 1993; Terry et al., 1998; Panchenko et al., 2000; Shan et al., 2000; Wagener et al., 2003). HO-2, a 36-kDa protein, is constitutively expressed in brain, testis, liver and endothelium (Maines et al., 1986; Trakshel et al., 1986; Sun et al., 1990; Zakhary et al., 1996). HO-3, although related to HO-2, is a product of a different gene, with a lesser ability than HO-2 to catalyze heme degradation (Jr McCoubrey et al., 1997). Several studies demonstrated that HO-1 and HO-2 can be detected in the fetal liver from an early stage of ontogeny (Sun and Maines, 1990; Watanabe et al., 2003).

Lipopolysaccharide (LPS) is a toxic component of cell walls of gram-negative bacteria and is widely present in the digestive tracts of humans and animals. Humans are constantly exposed to low levels of LPS through infection. Gastrointestinal distress and alcohol drinking often increase permeability of LPS from gastrointestinal tract into blood (Mathurin et al., 2000). Numerous studies showed that LPS upregulated the expression of HO-1 in adult liver (Rizzardini et al., 1994, 1998). A recent study found that tumor necrosis factor alpha (TNF-α) was involved in LPS-induced upregulation of HO-1 in mouse liver (Oguro et al., 2002).

On the other hand, mimicking maternal infection by exposing the pregnant rodents to LPS leads to adverse developmental outcome, including embryonic resorption, intra-uterine fetal death, intra-uterine growth restriction, and preterm labor and delivery (Rivera et al., 1998; Buhimschi et al., 2003). In the LPS model, TNF-α is the major mediator leading to intra-uterine fetal death and intra-uterine growth restriction (Xu et al., 2006a). In the present study, we aimed to investigate the effects of maternal LPS exposure on the expression of HO-1 in fetal liver. We found that maternally administered LPS upregulated the expression of HO-1 in fetal liver. Reactive oxygen species (ROS), rather than TNF-α or nitric oxide (NO), are involved in LPS-induced upregulation of HO-1 in fetal liver.

2. Materials and methods

2.1. Chemicals

Lipopolysaccharide (*Escherichia coli* LPS, serotype 0127:B8), alpha-phenyl-*N*-*t*-butylnitrone (PBN), aminoguanidine (AG) and pentoxifylline (PTX) were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were from Sigma or as indicated in the specified methods.

2.2. Animals and treatments

The ICR (CD-1) 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/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 0. The present study included four separate experiments.

Experiment 1. To investigate the effects of LPS on the expression of HO-1 in fetal liver, the pregnant mice were administered with LPS in two different modes. In mode A, the pregnant mice were intraperitoneally (i.p.) injected with LPS (75 μg/kg) on gestational day (gd) 17 and sacrificed at different time point (2 h, 12 h, 24 h or 48 h) after LPS. In mode B, the pregnant mice were i.p. injected with different doses of LPS (1, 10, 75 μg/kg) on gd 17 and sacrificed 24 h after LPS. Fetal livers were excised for Western blot analysis.

Experiment 2. To investigate the role of ROS on maternally administered LPS-induced upregulation of HO-1 in fetal liver the pregnant mice were i.p. injected with PBN (100 mg/kg), a free radical spin trapping agent, 30 min prior to LPS (75 μg/kg, i.p.) and sacrificed 24 h after LPS. Fetal livers were excised for measurements of GSH and thiobarbituric acid-reactive substance (TBARS) and Western blot analysis.

Experiment 3. To investigate the role of NO on maternally administered LPS-induced upregulation of HO-1 in fetal liver the pregnant mice were i.p. injected with AG (100 mg/kg), a selective inhibitor of inducible nitric oxide synthase (iNOS), 30 min prior to LPS (75 μg/kg, i.p.). Twelve pregnant mice in each group were sacrificed 6 h after LPS for analysis of nitrite plus nitrate in maternal serum and amniotic fluid. The remaining pregnant mice were sacrificed 24 h after LPS. Fetal livers were excised for Western blot analysis.

Experiment 4. To investigate the role of TNF-α on maternally administered LPS-induced upregulation of HO-1 in fetal liver the pregnant mice were i.p. injected with PTX (100 mg/kg), an inhibitor of TNF-α synthesis, 30 min before LPS. Twelve mice were sacrificed 1.5 h after LPS treatment for measurement of TNF-α in maternal serum and amniotic fluid. The remaining pregnant mice were sacrificed 24 h after LPS. Fetal livers were excised for Western blot analysis.

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. Western blot analysis

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

2.4. Measurement of GSH content

The GSH content in fetal livers was determined by the method of Griffith (1980). GSH values were expressed as nmol mg^{-1} protein. Protein content was measured according to the method of Lowry et al. (1951).

2.5. Lipid peroxidation assay

Lipid peroxidation in fetal liver was quantified by measuring TBARS as described previously (Ohkawa et al., 1979). TBARS values were expressed as nmol mg^{-1} protein. Protein content was measured according to the method of Lowry et al. (1951).

2.6. Analysis of nitrite plus nitrate in maternal serum and amniotic fluid

Nitrate plus nitrite, the stable end products of L-arginine-dependent nitric oxide synthesis, were measured using a colorimetric method based on the Griess reaction (Grisham et al., 1996).

2.7. Measurement for TNF-α in maternal serum and amniotic fluid

Maternal serum and amniotic fluid were collected at 1.5 h after maternal LPS administration. Commercial ELISA kits (R&D Systems Inc., Minneapolis, MN) were used to determine the level of TNF-α in maternal serum and amniotic fluid according to the manufacturer's protocol.

2.8. Statistical analysis

For Western blot studies, the densitometry unit of the control was assigned as 1. All quantified data were expressed as means ± S.E. at each point. ANOVA and the Student–Newmann–Keuls post hoc test were used to determine differences among different groups.

3. Results

3.1. Effects of maternal LPS exposure on the expression of HO-1 in fetal liver

To investigate the effects of maternal LPS exposure on the expression of HO-1 in fetal liver, a single dose of LPS (75 μg/kg, i.p.) were administered to the pregnant mice on gd 16 or gd 17. A single dose of LPS (75 μg/kg, i.p.) administered to the pregnant mice on gd 17 did not cause preterm labor and fetal death. As shown in Fig. 1, a basal expression of HO-1 was observed in fetal liver. The expression of HO-2, the constitutive form of HO, was also detected in fetal liver. When the pregnant mice were challenged with LPS, the expression of HO-1 in fetal liver was increased, beginning 2 h after LPS, being at the highest level 24 h after LPS treatment, and remaining elevated up to 48 h after LPS administration, whereas HO-2, the constitutive form, did not change at the various time points observed. To explore whether a dose–effect relationship existed, different doses of LPS (1, 10, 75 μg/kg, i.p.) were administered to the pregnant mice on gd 17. The expression of HO-1 in fetal liver was measured at 24 h after maternal LPS administration. As shown in Fig. 2, the expression of HO-1 in fetal liver was upregulated only at 75 μg/kg.

3.2. Role of ROS on maternally administered LPS-induced upregulation of HO-1 in fetal liver

To investigate the role of ROS on maternally administered LPS-induced upregulation of HO-1 in fetal liver, the pregnant mice were pretreated with PBN, a free radical spin trapping agent, at 30 min before maternal LPS administration. As expected, PBN pretreatment significantly attenuated LPS-induced lipid peroxidation and GSH depletion in fetal liver (Fig. 3). The effects of PBN on LPS-induced upregulation of HO-1 are presented in Fig. 4. Results showed that PBN alone did not influence the expression of HO-1 and HO-2 in fetal liver.

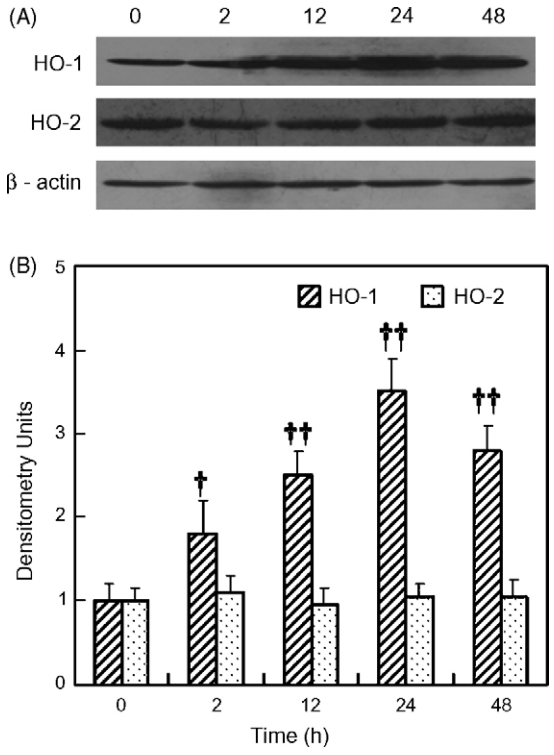

Fig. 1. The effects of maternally administered LPS on the expression of HO in fetal liver: time-course analysis. A single dose of LPS (75 μg/kg, i.p.) was administered to the pregnant mice. Fetal livers were excised at 2 h, 12 h, 24 h or 48 h after LPS. Western blot analysis was performed using anti-HO-1 and anti-HO-2 polyclonal antibodies. (A) A representative gel for HO-1 (upper panel) and HO-2 (lower panel) was shown. (B) Quantitative analysis of scanning densitometry on four samples from four different animals at each time point was performed. The densitometry unit of the control was assigned as 1. Data were expressed as means ± S.E. $^{\dagger\dagger}P < 0.01$ as compared with control group.

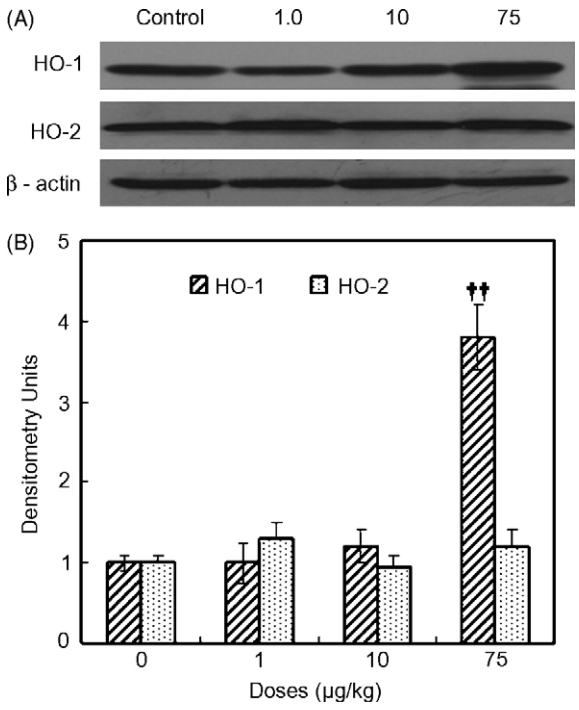

Fig. 2. The effects of maternally administered LPS on the expression of HO in fetal liver: dose–effect relationship. Different doses of LPS (1, 10, 75 μg/kg, i.p.) were administered to the pregnant mice on gd 17. Fetal livers were excised at 24 h after LPS administration. Western blot analysis was performed using anti-HO-1 and anti-HO-2 polyclonal antibodies. (A) A representative gel for HO-1 (upper panel) and HO-2 (lower panel). (B) Quantitative analysis of scanning densitometry on four samples from four different animals each dose was performed. The densitometry unit of the control was assigned as 1. Data were expressed as means ± S.E. $^{\dagger\dagger}P < 0.01$ as compared with control group.

Interestingly, PBN pretreatment significantly attenuated LPS-induced upregulation of HO-1 in fetal liver. PBN pretreatment had no effect on the expression of HO-2 in fetal liver.

3.3. Role of nitric oxide on maternally administered LPS-induced upregulation of HO-1 in fetal liver

To investigate the role of NO on maternally administered LPS-induced upregulation of HO-1 in fetal liver, the pregnant mice were pretreated with AG, a selective inhibitor of iNOS, at 30 min before maternal LPS administration. As shown in Fig. 5, LPS had no effect on nitrite plus nitrate production in amniotic fluid, whereas LPS slightly increased nitrite plus nitrate level in maternal serum. AG pretreatment significantly attenuated LPS-evoked NO production in maternal serum. However, AG had no effect on LPS-induced upregulation of HO-1 in fetal liver (Fig. 6). Similarly, AG pretreatment did not influence the expression of HO-2 in fetal liver.

3.4. Role of TNF-α on maternally administered LPS-induced upregulation of HO-1 in fetal liver

To investigate the role of TNF-α on maternally administered LPS-induced upregulation of HO-1 in fetal liver, the pregnant mice were pretreated with PTX (100 mg/kg, i.p.), an inhibitor of TNF-α synthesis, at 30 min before maternal LPS administration. As expected, PTX pretreatment significantly attenuated LPS-induced increase in TNF-α in maternal serum and amniotic fluid (Fig. 7). However, PTX pretreatment had no effect on LPS-induced upregulation of HO-1 in fetal liver. Similarly, PTX pretreatment did not influence the expression of HO-2 in fetal liver (Fig. 8).

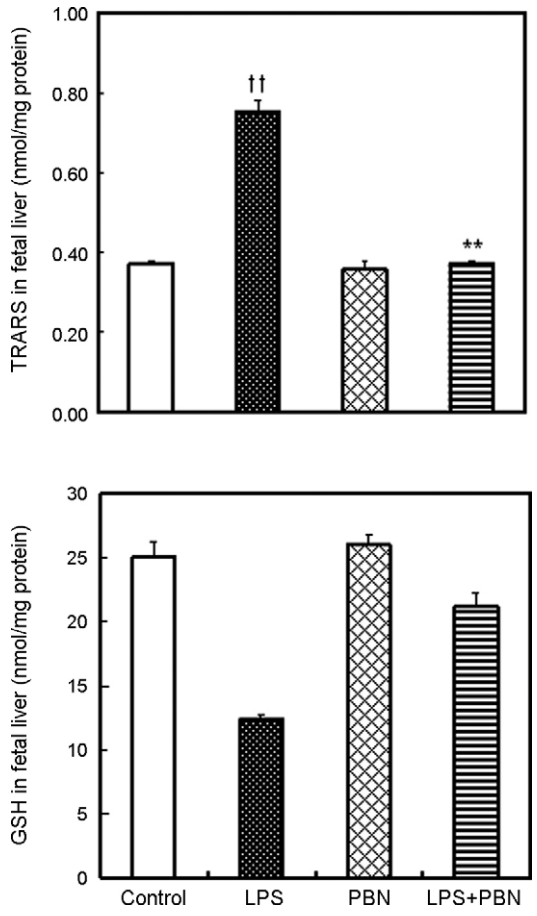

Fig. 3. The effects of PBN on maternally administered LPS-induced lipid peroxidation and GSH depletion in fetal liver. A single dose of PBN (100 mg/kg, i.p.) was injected to the pregnant mice 30 min prior to LPS (75 μg/kg, i.p.). Fetal livers were excised and homogenized 24 h after LPS. The levels of TBARS and GSH in fetal liver were analyzed. Data were expressed as means ± S.E. ($n = 12$). $^{\dagger\dagger}P < 0.01$ as compared with control group. $^{**}P < 0.01$ as compared with LPS group.

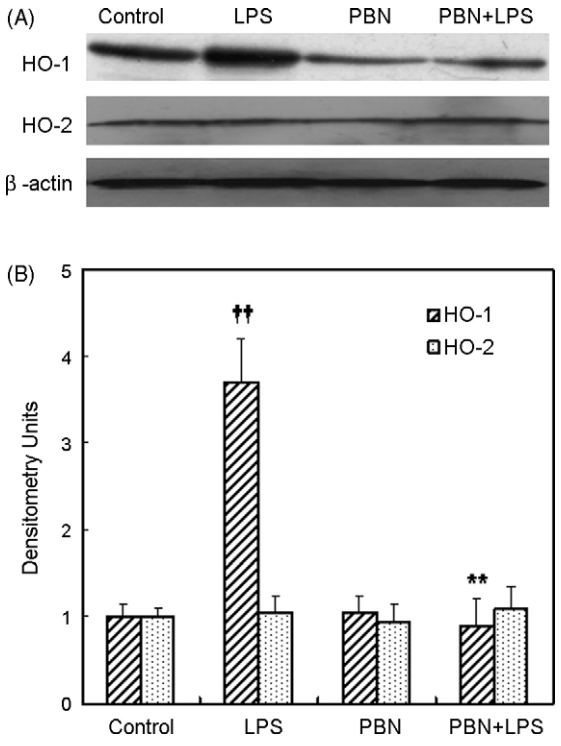

Fig. 4. The effects of PBN on maternally administered LPS-induced upregulation of HO-1 in fetal liver. A single dose of PBN (100 mg/kg, i.p.) was injected to the pregnant mice 30 min prior to LPS (75 μg/kg, i.p.). Fetal livers were excised 24 h after LPS. Western blot analysis was performed using anti-HO-1 and anti-HO-2 polyclonal antibodies. (A) A representative gel for HO-1 (upper panel) and HO-2 (lower panel). (B) Quantitative analysis of scanning densitometry on four samples from four different animals at each treatment was performed. The densitometry unit of the control was assigned as 1. Data were expressed as means ± S.E. $^{\dagger\dagger}P < 0.01$ as compared with control group. $^{**}P < 0.01$ as compared with LPS group.

4. Discussion

Heme oxygenase (HO) catalyzes the rate-limiting step in the degradation of heme to yield equimolar amounts of biliverdin, carbon monoxide, and iron. HO-1 is the only inducible one. Previous studies have demonstrated that LPS upregulates the expression of HO-1 in adult mouse liver (Rizzardini et al., 1994, 1998). In the present study, we showed that a basal expression of HO-1 and HO-2 was observed in fetal liver. When the pregnant mice were challenged with LPS, the expression of HO-1 in fetal liver was upregulated, beginning at 2 h after LPS, being at the highest level 24 h after LPS treatment, and remaining elevated up to 48 h after LPS administration, whereas HO-2, the constitutive form, did not change at the various time points observed.

ROS are important mediators in regulation of genes and signal transduction pathways (Allen and Tresini, 2000). LPS, a potent activator for macrophages, stimulates Kupffer cells to generate ROS (Bautista et al., 1990). Several studies shown that LPS increased nitrotyrosine, a marker for nitric oxide and peroxynitrite ($ONOO^-$) formation, in macrophage-rich organs, such as liver (Bian and Murad, 2001; Ottesen et al., 2001). ROS are involved in LPS-induced upregulation of HO-1 in macrophage (Camhi et al., 1998). Indeed, macrophages (Kupffer cells) are present in fetal liver. A recent study found that murine fetal liver expresses high levels of TLR-4 mRNA (Harju et al., 2001). In vitro studies indicated that fetal Kupffer cells express mature macrophage function in early gestation (Kutteh et al., 1991). The present study showed that maternal LPS exposure resulted in lipid peroxidation and GSH depletion in fetal liver. To determine the role of ROS

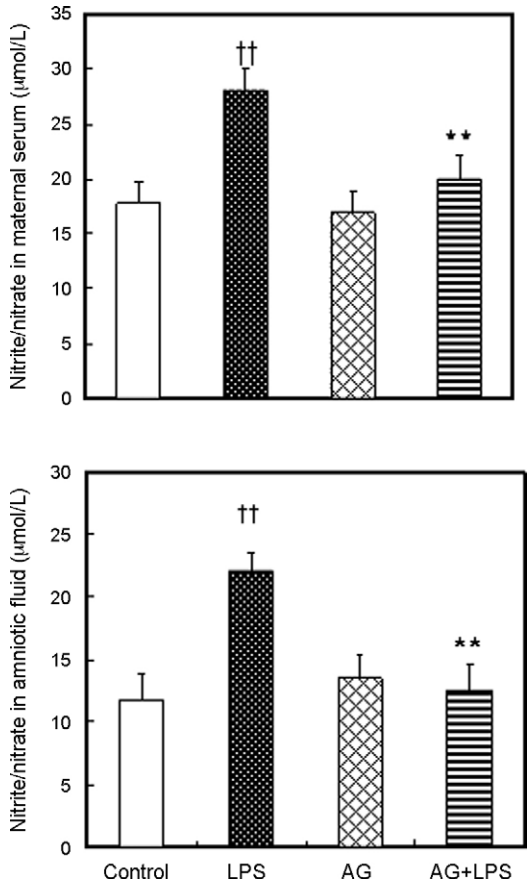

Fig. 5. The effects of maternal LPS exposure on NO production in maternal serum and amniotic fluid. The pregnant mice were injected with LPS (75 μg/kg, i.p.) on gd 17. In AG group, the pregnant mice were pretreated with AG (100 mg/kg, i.p.) before LPS. Maternal serum and amniotic fluid were collected 6 h after LPS. The levels of Nitrite plus nitrate maternal serum and amniotic fluid were analyzed via the Griess reaction as described in Section 2. Data were expressed as means ± S.E. ($n = 12$). $^{††}P < 0.01$ as compared with control group. $^{**}P < 0.01$ as compared with LPS group.

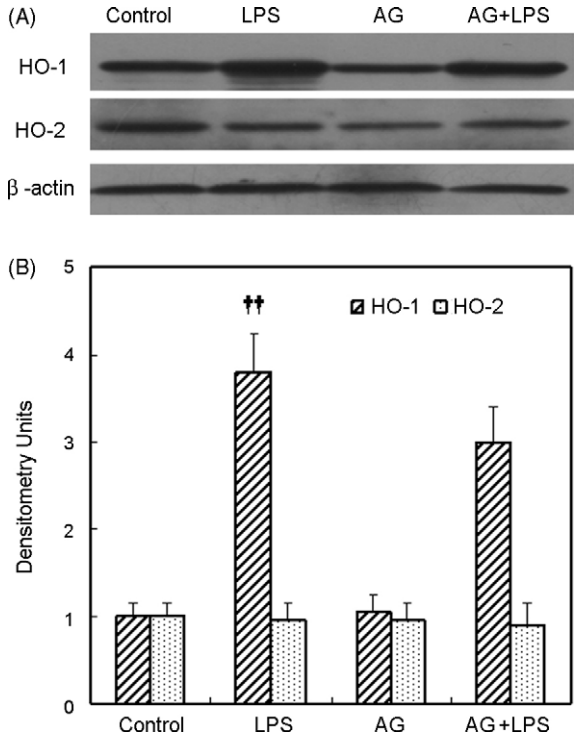

Fig. 6. The effects of AG on maternally administered LPS-induced upregulation of HO-1 in fetal liver. A single dose of AG (100 mg/kg, i.p.) was injected to the pregnant mice 30 min prior to LPS (75 μg/kg, i.p.). Fetal livers were excised 24 h after LPS. Western blot analysis was performed using anti-HO-1 and anti-HO-2 polyclonal antibodies. (A) A representative gel for HO-1 (upper panel) and HO-2 (lower panel). (B) Quantitative analysis of scanning densitometry on four samples from four different animals at each treatment was performed. The densitometry unit of the control was assigned as 1. Data were expressed as means ± S.E. $^{††}P < 0.01$ as compared with control group.

on maternally administered LPS-induced upregulation of HO-1 in fetal liver, PBN, a free radical spin trapping agent, was used to eliminate LPS-evoked ROS. As expected, maternally administered PBN significantly attenuated LPS-induced lipid peroxidation and GSH depletion in fetal liver, suggesting that maternally administered PBN was effective in the in vivo trapping of free radicals. Consistent with its antioxidant effect, PBN almost completely blocked LPS-induced upregulation of HO-1 in fetal liver. These results indicate that ROS mediate LPS-induced upregulation of HO-1 in fetal liver. The mechanism for ROS-mediated upregulation of HO-1 remains unclear. Several in vitro studies demonstrated that LPS upregulated HO-1 gene transcription in part through stimulating ROS production, which initiated signal transduction pathway leading to the activation of either activator protein (AP)-1 or nuclear factor kappa B (NF-κ) (Kurata et al., 1996; Camhi et al., 1998). A recent study indicated that GSH depletion aggravated LPS-induced upregulation of HO-1 (Srisook et al., 2005). The present study showed that maternally administered LPS significantly decreased the level of GSH in fetal liver. Thus, the present data do not exclude the involvement of GSH depletion in LPS-induced upregulation of HO-1 in fetal liver.

LPS is an inducible nitric oxide synthase (iNOS) inducer. Several studies showed that maternal LPS exposure increased the expression of iNOS in decidual and myometrial cells and NO production in decidual and uterine (Athanassakis et al., 1999; Ogando et al., 2003). Indeed, NO is a HO-1 inducer (Kitamura et al., 1998; Datta and Lianos, 1999). Several studies showed that NO was mediated in LPS-induced upregulation of HO-1 in macrophages (Srisook and Cha, 2004, 2005; Srisook et

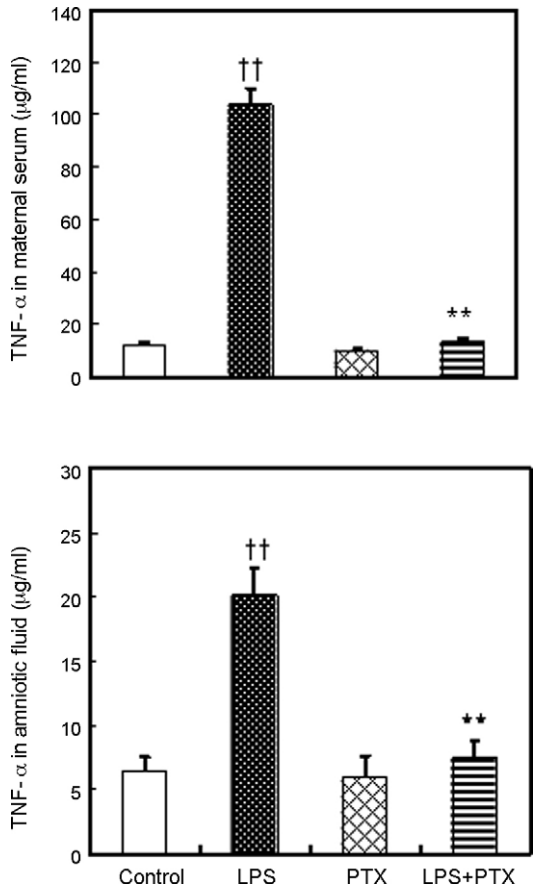

Fig. 7. The effects of maternal LPS exposure on TNF-α level in maternal serum and amniotic fluid. The pregnant mice were injected with LPS (75 μg/kg, i.p.) on gd 17. In PTX group, the pregnant mice were administered with PTX (100 mg/kg, i.p.) 30 min prior to LPS. The level of TNF-α in maternal serum and amniotic fluid was measured at 1.5 h after LPS using ELISA. Data were expressed as means ± S.E. of twelve mice in each point. $^{††}P < 0.01$ as compared with control group. $^{**}P < 0.01$ as compared with LPS group.

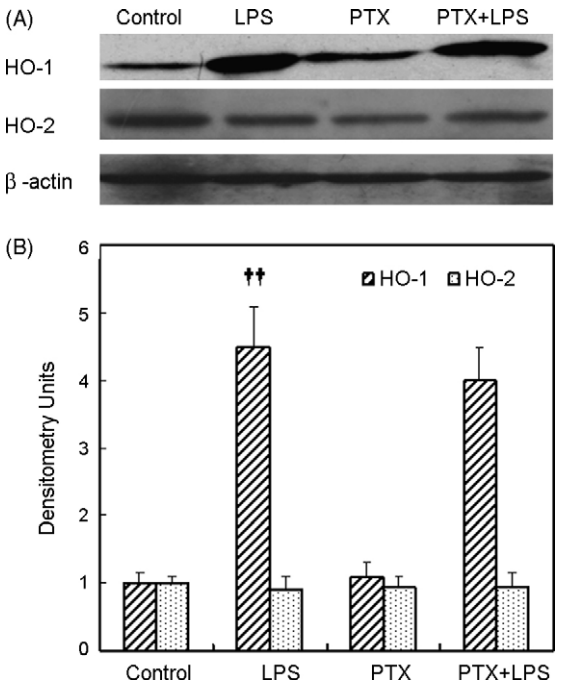

Fig. 8. The effects of PTX on maternally administered LPS-induced upregulation of HO-1 in fetal liver. A single dose of PTX (100 mg/kg, i.p.) was injected to the pregnant mice 30 min prior to LPS (75 μg/kg, i.p.). Fetal livers were excised 24 h after LPS. Western blot analysis was performed using anti-HO-1 and anti-HO-2 polyclonal antibodies. (A) A representative gel for HO-1 (upper panel) and HO-2 (lower panel). (B) Quantitative analysis of scanning densitometry on four samples from four different animals at each treatment was performed. The densitometry unit of the control was assigned as 1. Data were expressed as means ± S.E. $^{††}P < 0.01$ as compared with control group.

al., 2005). The present study showed that maternal LPS exposure significantly increased the level of nitrite plus nitrate in maternal serum and amniotic fluid. However, AG, a selective iNOS inhibitor, had little effect on LPS-induced upregulation of HO-1 in fetal liver, although it significantly attenuated LPS-evoked NO production in maternal serum and amniotic fluid. These results suggest that LPS-induced upregulation of HO-1 in fetal liver is independent of NO production.

Numerous studies demonstrated that proinflammatory cytokines, such as TNF-α or interleukin (IL)-1β, were involved in LPS-induced upregulation of HO-1 (Rizzardini et al., 1998; Oguro et al., 2002; Song et al., 2003). Several studies showed that LPS stimulated TNF-α and IL-1β in amniotic fluid and placenta (Bell et al., 2004; Gayle et al., 2004). Recently, we found that maternally administered LPS significantly increased the levels of TNF-α, IL-1β and IL-6 in fetal liver (Xu et al., 2007a, 2008). To investigate the role of TNF-α in LPS-induced upregulation of HO-1 in fetal liver, the pregnant mice were pretreated with PTX, an inhibitor of TNF-α synthesis. As expected, PTX pretreatment significantly inhibited LPS-induced release of TNF-α in maternal serum and amniotic fluid. However, PTX pretreatment had no effect on LPS-induced upregulation of HO-1 in fetal liver, suggesting that LPS-induced upregulation of HO-1 in fetal liver is also independent of TNF-α production.

It has been demonstrated that maternal LPS exposure results in intra-uterine fetal death, growth development retardation, preterm delivery, and aberrant brain development and neurological disorders in fetuses (Xu et al., 2006b). An earlier study showed that perinatal LPS exposure downregulated the expression of pregnane X receptor and cytochrome P4503a11 in fetal liver (Xu et

al., 2005b). Previous studies thought that LPS-induced developmental toxicity is a maternally mediated event (Leazer et al., 2002). The present study demonstrated that prenatal LPS exposure upregulated the expression of HO-1 in fetal liver, which provides additional direct evidence for oxidative stress in fetal tissues. Importantly, LPS-induced upregulation of HO-1 in fetal liver was inhibited by radical trapping agent PBN. In addition, several earlier studies showed that antioxidants, such as melatonin, ascorbic acid and *N*-acetylcysteine, protected rodents against LPS-induced intra-uterine fetal death, growth retardation, preterm delivery and perinatal brain injury (Buhimschi et al., 2003; Xu et al., 2005a; Chen et al., 2006a,b; Wang et al., 2007). Therefore, antioxidants may be worth exploring as potential embryoprotective agents for clinical use during maternal infections with bacterial or viral agents.

In summary, the present study indicates that maternally administered LPS significantly upregulated the expression of HO-1 in fetal liver. ROS, rather than TNF-α or nitric oxide, are involved in LPS-induced up-regulation of HO-1 in fetal liver. These results provide new evidence that maternal LPS exposure results in oxidative stress in fetuses, which may contribute to LPS-induced developmental toxicity.

Acknowledgements

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