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Ascorbic acid protects against lipopolysaccharide-induced intra-uterine fetal death and intra-uterine growth retardation in mice

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Abstract

Lipopolysaccharide (LPS) has been associated with adverse developmental outcomes including embryonic resorption, intra-uterine fetal death (IUFD), intra-uterine growth retardation (IUGR) and preterm labor. Reactive oxygen species (ROS) mediate LPS-induced developmental toxicity. Ascorbic acid is an antioxidant. In the present study, we investigated the effect of ascorbic acid on LPS-induced IUFD and IUGR in mice. All ICR pregnant mice except controls received an intraperitoneal (75 μg/kg, i.p.) injection of LPS daily on gd 15–17. The experiment was carried out in three different modes. In mode A, the pregnant mice were pretreated with a single dose (500 mg/kg, i.p.) of ascorbic acid before LPS. In mode B, the pregnant mice were administered with a single dose (500 mg/kg, i.p.) of ascorbic acid at 3 h after LPS. In mode C, the pregnant mice were administered with 500 mg/kg (i.p.) of ascorbic acid at 30 min before LPS, followed by additional dose (500 mg/kg, i.p.) of ascorbic acid at 3 h after LPS. The number of live fetuses, dead fetuses and resorption sites was counted on gd 18. Live fetuses in each litter were weighed. Crown-rump and tail lengths were examined and skeletal development was evaluated. Results showed that maternally administered LPS significantly increased fetal mortality, decreased fetal weight and crown-rump and tail lengths of live fetuses, and retarded skeletal ossification in caudal vertebrae, anterior and posterior phalanges, and supraoccipital bone. LPS-induced IUFD and IUGR were associated with lipid peroxidation and GSH depletion in maternal liver, placenta and fetal liver. Pre-treatment with ascorbic acid significantly attenuated LPS-induced lipid peroxidation, decreased fetal mortality, and reversed LPS-induced fetal growth and skeletal development retardation. By contrast to pre-treatment, post-treatment with ascorbic acid had less effect on LPS-induced IUFD, although post-treatment significantly attenuated LPS-induced lipid peroxidation and reversed LPS-induced fetal growth and skeletal development retardation. Furthermore, post-treatment with ascorbic acid reduced the protective effects of pre-treatment on LPS-induced IUFD. All these results suggest that pre-treatment with ascorbic acid protected against LPS-induced fetal death and reversed LPS-induced growth and skeletal development retardation via counteracting LPS-induced oxidative stress, whereas post-treatment had less effect on LPS-induced IUFD.

Keywords: Antioxidant; Ascorbic acid; Lipopolysaccharide; Intra-uterine fetal death; Intra-uterine growth retardation

Abbreviations: AA, ascorbic acid; GSH, glutathione; iNOS, inducible nitric oxide synthase; IUFD, intra-uterine fetal death; IUGR, intra-uterine growth retardation; LPS, lipopolysaccharide; NF-kB, nuclear factor-kB; NO, nitric oxide; $O_2^{\bullet -}$, superoxide anion; OR, odds ratios; ROS, reactive oxygen species; TBARS, thiobarbituric acid-reactive substance

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1. Introduction

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 (Fukui et al., 1991). LPS has been associated with adverse developmental outcome, including embryonic resorption, intra-uterine fetal death (IUFD), intra-uterine growth retardation (IUGR) and preterm labor in animals (O'Sullivan et al., 1988; Collins et al., 1994). However, the exact mechanism of LPS-induced developmental toxicity remained unclear.

Silver et al. (1995) reported that pregnant C3H/HeN mice injected with LPS showed an increase in decidual eicosanoid production and COX_2 expression, followed by a dose-dependent increase in embryo death. Furthermore, COX_2 suppressors decreased IUFD and prevented LPS-induced preterm delivery (Sakai et al., 2001), indicating that eicosanoids might be important mediators of LPS-induced adverse developmental outcome. On the other hand, a recent study showed that maternal LPS exposure significantly increased inducible nitric oxide synthase (iNOS) expression in decidual and myometrial cells and nitric oxide (NO) production in decidual and uterine (Ogando et al., 2003). In addition, aminoguanidine (AG), a specific inhibitor of iNOS activity, reversed LPS-induced embryonic resorption and abortion (Athanassakis et al., 1999). These results suggest that NO fulfils a fundamental role in LPS-induced embryonic resorption and abortion. However, recent unpublished results from our laboratory show that AG had little effect on LPS-induced IUFD and IUGR.

Numerous studies indicated that maternal LPS exposure increased TNF-α production in maternal serum and amniotic fluid (Gayle et al., 2004). Our earlier report showed that a single dose of LPS upregulated TNF-α mRNA expression in mouse placenta (Chen et al., 2005). Mother-derived TNF-α has been associated with LPS-induced preterm labor and delivery (Leazer et al., 2002). However, a recent study found that LPS-induced IUFD was not blocked by treatment with anti-TNF antibody that inhibited LPS-induced TNF-α production in pregnant females (Kohmura et al., 2000). Another important study by Casado et al. (1997), comparing difference in plasma TNF-α levels between in mother and in fetus, showed that less than 7% TNF-α was detected in the corresponding fetal serum although serum from mother treated with LPS exhibited a significant increase in TNF-α levels, suggesting a restricted permeability of placenta to proinflammatory cytokines.

LPS stimulates macrophages to generate reactive oxygen species (ROS) and increases nitrotyrosine, a marker for $O_2^{\bullet -}$, NO and $ONOO^-$ formation, in macrophage-rich organs (Bautista et al., 1990). A recent study showed that *N*-acetylcysteine, a glutathione (GSH) precursor and direct antioxidant, protected fetal death and preterm labor induced by maternal inflammation (Buhimschi et al., 2003), suggesting that ROS may mediate LPS-induced developmental toxicity. Ascorbic acid is an antioxidant and has been demonstrated to be effective on preventing fetal malformation in experimental diabetic pregnancy (Cederberg et al., 2001). In this study, we investigated the effect of ascorbic acid on LPS-induced IUFD and IUGR in ICR mice. The present results indicated that pre-treatment with ascorbic acid protected against LPS-induced fetal death and reversed LPS-induced fetal growth and skeletal development retardation via counteracting LPS-induced oxidative stress, whereas post-treatment with ascorbic acid had less effect on LPS-induced IUFD.

2. Materials and methods

2.1. Chemicals

Lipopolysaccharide (*Escherichia coli* LPS, serotype 0127:B8) and ascorbic acid (AA) were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were obtained from Sigma if not otherwise stated.

2.2. Animals and treatments

The ICR mice (8–10 week-old; male mice: 30–32 g; female mice: 26–28 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 (gd) 0. The present study included two separate experiments.

2.2.1. Experiment 1

The timed pregnant mice were divided randomly into six groups. All pregnant mice except controls received an intraperitoneal (75 μg/kg, i.p.) injection of LPS on gd 15–17. The experiment was carried out in three different modes. In mode A, the pregnant mice were pretreated with a single dose (500 mg/kg, i.p.) of ascorbic acid before LPS administration. In mode

B, the pregnant mice were administered with a single dose (500 mg/kg, i.p.) of ascorbic acid at 3 h after LPS treatment. In mode C, the pregnant mice were administered with two doses of ascorbic acid in 24 h, one (500 mg/kg) injected at 30 min before LPS and the other (500 mg/kg) injected 3 h after LPS. The saline- and AA-treated pregnant mice served as controls. All dams were sacrificed on gd 18 and gravid uterine weights were recorded. For each litter, the number of live fetuses, dead fetuses and resorption sites was counted. Live fetuses in each litter were weighed. Crown-rump and tail lengths were measured. All fetuses were then stored in ethanol a minimum of 2 weeks for subsequent skeletal evaluation.

2.2.2. Experiment 2

The timed pregnant mice were divided randomly into six groups. All pregnant mice except controls received an intraperitoneal (75 μg/kg, i.p.) injection of LPS on gd 15. Ascorbic acid treatments were the same as in experiment 1. In this experiment, dams were sacrificed at 6 h after LPS treatment. Maternal liver, placenta and fetal liver samples were excised for measurement of TBARS and GSH contents.

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. Skeletal examination and evaluation

The fetuses stored in ethanol were cleared from skin, viscera and adipose tissue. Fetuses were then incubated in acetone overnight and subsequently macerated and stained with alizarin red S for 2 d. After an overnight incubation in 70% ethanol/glycerol/benzyl alcohol, the fetuses were stored in glycerol until examination. Skeletal evaluation included determination of the degree ossification of the phalanges, metacarpals, vertebrae, sternatrae and skull. The size of ossification of the supraoccipital was scored (1 = well ossified, 4 = completely unossified).

2.4. Determination of glutathione content

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

2.5. Lipid peroxidation assay

Lipid peroxidation was quantified by measuring thiobarbituric acid-reactive substance (TBARS) as described previously (Ohkawa et al., 1979). Tissue was homogenized in nine volumes of 50 mmol/L Tris–HCl buffer (pH 7.4) containing 180 mmol/L KCl, 10 mmol/L EDTA, and 0.02% butylated hydroxytoluene. To 0.2 ml of the tissue homogenate, 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid, 1.5 ml of 0.9% thiobarbituric acid, and 0.6 ml of distilled water were added and vortexed. The reaction mixture was placed in a water bath at 95 °C for 1 h. After cooling on ice, 1.0 ml of distilled water and 5.0 ml of butanol/pyridine mixture (15:1 v/v) were added and vortexed. After centrifugation at $10{,}000 \times g$ 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.

2.6. Statistical analysis

Quantified data were expressed as means ± S.E.M. at each point. Binomial data were analyzed using X^2 analysis or Fisher's exact test where appropriate. ANOVA and the Student–Newmann–Keuls post hoc test were used to determine differences between the treated animals and the control and statistical significance.

3. Results

3.1. Effects of ascorbic acid on LPS-induced IUFD

The effects of ascorbic acid (AA) on LPS-induced IUFD are presented in Table 1. Results showed that the incidence of resorptions did not differ among different groups. LPS and ascorbic acid exhibited no obvious maternal side effects (data not shown). The incidence of fetal death was 63.2% (98/155) in LPS group, significantly higher than 0.4% (1/232) in control group. Fetal mortality was 0.8% (1/121) in AA group, suggesting that AA alone did result in fetal death. The effects of ascorbic acid on LPS-induced IUFD depend on the schedule of treatment. Fetal mortality was 23.6% (34/144) in pre-treatment group, significantly lower than 63.2% in LPS group (OR: 0.18; 95% CI: 0.10–0.31). Fetal mortality was 48.8% (62/127) in post-treatment group, significantly lower than 63.2% in LPS group (OR: 0.55; 95% CI: 0.33–0.92). However, post-treatment with AA was less effective than pre-treatment (post-treatment versus pre-treatment, OR: 3.09; 95% CI: 1.78–5.37). Furthermore, post-treatment reduced the protective effects of pre-treatment (pre- and post-treatment versus pre-treatment, OR: 2.29; 95% CI: 1.42–3.70).

Table 1
The effects of AA on LPS-induced fetal death

Treatments	Litters	Gestational sacs	Fetal resorption	Live fetuses	Dead fetuses	Fetal mortality[a] % (95% C.I.)
Control	21	243	11	231	1	0.4 (0, 1.3)
AA (500 mg/kg)	10	128	6	121	1	0.8 (0.6, 1.0)
LPS (75 μg/kg)	12	161	6	57	98	63.2 (55.6, 70.82)††
AA (pre-, 500 mg/kg) + LPS (75 μg/kg)	11	150	6	110	34	23.6 (16.7, 30.5)**
LPS (75 μg/kg) + AA (post-, 500 mg/kg)	10	132	5	65	62	48.8 (40.1, 57.5)*
LPS (75 μg/kg) + AA (pre- + post-, 500 + 500 mg/kg)	14	187	6	106	75	41.4 (34.3, 48.6)**,§§

[a] Fetal mortality (%) = ·(dead fetuses/live fetuses + dead fetuses) × 100.
* Significantly different from LPS group, $P < 0.05$.
** Significantly different from LPS group, $P < 0.01$.
†† Significantly different from control, $P < 0.01$.
§§ Significantly different from pre-treatment with AA, $P < 0.01$.

3.2. Effects of ascorbic acid on LPS-induced IUGR

The effects of ascorbic acid on LPS-induced IUGR are presented in Table 2. Results showed that maternally administered LPS significantly decreased fetal weight and crown-rump and tail lengths. All treatments with AA significantly attenuated LPS-induced decrease in fetal weight and crown-rump and tail lengths. Placental size was statistically indistinguishable among different groups (data not shown).

3.3. Effects of ascorbic acid on LPS-induced skeletal development retardation

The effects of AA on LPS-induced skeletal development retardation are presented in Table 3. The fetal skeleton of the LPS-treated mice, compared with the control group, exhibited fewer ossification centers in caudal vertebrae, anterior and posterior phalanges. In addition, LPS also retarded supraoccipital ossification. All treatments with AA significantly attenuated LPS-induced skeletal development retardation.

3.4. Effects of ascorbic acid on LPS-induced lipid peroxidation

Lipid peroxidation was quantified by measuring TBARS. The effects of AA on LPS-induced lipid peroxidation are presented in Table 4. Results showed that a single dose (75 μg/kg) of LPS caused an increase in TBARS levels by 90.1% in maternal liver, 87.5% in placenta, and 78.3% in fetal liver. Both pre- and post-treatments with AA significantly attenuated LPS-evoked increase in TBARS level in maternal liver, placenta and fetal liver. However, the protective effects of pre-treatment on LPS-induced lipid peroxidation in maternal liver and placenta were counteracted when the pregnant mice were administered with additional dose of AA at 3 h after LPS treatment.

3.5. Effects of ascorbic acid on LPS-induced GSH depletion

The effects of ascorbic acid on GSH content are presented in Table 5. Results showed that LPS decreased

Table 2
The effects of AA on LPS-induced intra-uterine growth retardation

Treatments	Litters	Live fetuses	Crown-rump length (mm, $\bar{x} \pm S$)	Tail length (mm, $\bar{x} \pm S$)	Fetal weight (g, $\bar{x} \pm S$)
Control	21	231	23.5 ± 0.54	11.7 ± 0.23	1.30 ± 0.05
AA (500 mg/kg)	10	129	22.8 ± 0.50	11.5 ± 0.20	1.31 ± 0.05
LPS (75 μg/kg)	10	57	19.8 ± 0.56††	9.4 ± 0.46††	1.02 ± 0.05††
LPS (75 μg/kg) + AA (pre-, 500 mg/kg)	11	110	22.6 ± 1.23**	11.3 ± 0.49**	1.19 ± 0.08**
LPS (75 μg/kg) + AA (post-, 500 mg/kg)	10	65	22.0 ± 0.82**	11.5 ± 0.43**	1.17 ± 0.11**
LPS (75 μg/kg) + AA (pre- + post-, 500 + 500 mg/kg)	14	106	22.6 ± 1.40**	11.1 ± 0.52**	1.15 ± 0.14**

** Significantly different from LPS group, $P < 0.01$.
†† Significantly different from control, $P < 0.01$.

Table 3
The effects of ascorbic acid on LPS-induced skeletal development retardation

	Control ($n = 21$)	AA ($n = 10$)	LPS ($n = 12$)	LPS + AA (pre-) ($n = 11$)	LPS + AA (post-) ($n = 10$)	LPS + AA (pre- + post-) ($n = 14$)
Number examined						
Live fetuses	231	121	57	110	65	106
Scores						
Supraoccipital bone[a]	1.02 ± 0.01	1.10 ± 0.03	$2.02 \pm 0.25^{\dagger\dagger}$	$1.20 \pm 0.20^{**}$	$1.32 \pm 0.21^{**}$	$1.12 \pm 0.10^{**}$
Number ossified						
Sternum	6.00 ± 0.00	5.94 ± 0.25	$5.87 \pm 0.26^{\dagger}$	6.00 ± 0.00	6.00 ± 0.00	5.97 ± 0.09
Metacarpus	4.00 ± 0.00	3.95 ± 0.02	3.88 ± 0.29	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
Anterior phalanx	4.00 ± 0.00	3.95 ± 0.10	$3.08 \pm 1.42^{\dagger\dagger}$	3.95 ± 0.07	4.00 ± 0.00	$3.95 \pm 0.10^{*}$
Metatarsus	4.98 ± 0.01	4.60 ± 0.30	$4.56 \pm 0.39^{\dagger\dagger}$	4.73 ± 0.54	$4.93 \pm 0.14^{*}$	$4.85 \pm 0.18^{*}$
Posterior phalanx	4.95 ± 0.02	3.80 ± 0.74	$2.99 \pm 2.09^{\dagger\dagger}$	$4.91 \pm 0.18^{**}$	$4.82 \pm 0.36^{*}$	$4.07 \pm 0.92^{\S}$
Caudal vertebrae	6.30 ± 0.21	5.50 ± 0.85	$3.67 \pm 0.91^{\dagger\dagger}$	$5.14 \pm 1.31^{**}$	$5.36 \pm 1.54^{**}$	$4.90 \pm 0.82^{**}$

[a] Supraoccipital bone scores—1: well ossified, 4: completely unossified.
[*] Significantly different from LPS group, $P < 0.05$.
[**] Significantly different from LPS group, $P < 0.01$.
[†] Significantly different from control, $P < 0.05$.
[††] Significantly different from control, $P < 0.01$.
[§] Significantly different from pre-treatment with AA, $P < 0.05$.

Table 4
The effects of AA on LPS-induced lipid peroxidation

Treatments	TBARS (nmol/mg protein)		
	Maternal liver	Placenta	Fetal liver
Control	0.81 ± 0.27	0.40 ± 0.07	1.43 ± 0.23
AA (500 mg/kg)	0.80 ± 0.25	0.42 ± 0.06	1.45 ± 0.19
LPS (75 μg/kg)	$1.54 \pm 0.33^{\dagger\dagger}$	$0.75 \pm 0.16^{\dagger\dagger}$	$2.55 \pm 0.25^{\dagger\dagger}$
AA (pre-, 500 mg/kg) + LPS (75 μg/kg)	$1.12 \pm 0.18^{**}$	$0.49 \pm 0.11^{**}$	$1.91 \pm 0.25^{**}$
LPS (75 μg/kg) + AA (post-, 500 mg/kg)	$1.21 \pm 0.18^{*}$	$0.51 \pm 0.09^{**}$	$2.22 \pm 0.21^{*}$
LPS (75 μg/kg) + AA (pre- + post-, 500 + 500 mg/kg)	$1.72 \pm 0.47^{\S\S}$	$1.72 \pm 0.54^{**,\S\S}$	$2.09 \pm 0.23^{**}$

[*] Significantly different from LPS group, $P < 0.05$.
[**] Significantly different from LPS group, $P < 0.01$.
[††] Significantly different from control, $P < 0.01$.
[§§] Significantly different from pre-treatment with AA, $P < 0.01$.

Table 5
The effects of AA on LPS-induced GSH depletion

Treatments	GSH content (nmol/mg protein)		
	Maternal liver	Placenta	Fetal liver
Control	34.4 ± 1.8	6.20 ± 1.60	25.1 ± 3.9
AA (500 mg/kg)	35.0 ± 2.0	6.02 ± 1.71	24.9 ± 3.8
LPS (75 μg/kg)	$22.5 \pm 2.1^{\dagger\dagger}$	$3.70 \pm 1.60^{\dagger\dagger}$	$12.4 \pm 1.2^{\dagger\dagger}$
LPS (75 μg/kg) + AA (pre-, 500 mg/kg)	$15.5 \pm 1.4^{**}$	$1.30 \pm 0.70^{**}$	$4.7 \pm 1.0^{**}$
LPS (75 μg/kg) + AA (post-, 500 mg/kg)	$17.3 \pm 2.8^{**}$	$1.45 \pm 0.42^{**}$	9.7 ± 4.4
LPS (75 μg/kg) + AA (pre- + post-, 500 + 500 mg/kg)	$17.9 \pm 3.7^{**}$	$0.66 \pm 0.21^{**,\S}$	$5.9 \pm 2.1^{**}$

[**] Significantly different from LPS group, $P < 0.01$.
[††] Significantly different from control, $P < 0.01$.
[§] Significantly different from pre-treatment with AA, $P < 0.05$.

GSH content by 34.6% in maternal liver, 40.3% in placenta, and 50.6% in fetal liver. All treatments with ascorbic acid significantly aggravated LPS-induced GSH depletion in maternal liver, placenta and fetal liver.

4. Discussion

The present study investigated the effects of LPS on IUFD and IUGR in mice. Results indicated that maternally administered LPS on gd 15–17 resulted in 63.2% fetal death. In addition, LPS significantly reduced fetal weight and crown-rump and tail lengths and retarded skeletal ossification in caudal vertebrae, anterior and posterior phalanges, and supraoccipital bone. These results are in agreement with earlier work of Rivera et al. (1998), in which administration with 100 μg/kg LPS to rats on gd 14–20 caused 43% fetal death and reduced the size of the surviving fetuses.

To this date, the exact mechanism for LPS-induced IUFD and IUGR remained unclear. Previous study demonstrated that LPS stimulates macrophages to generate ROS and increases nitrotyrosine, a marker for $O_2^{\bullet -}$, NO and $ONOO^-$ formation, in macrophage-rich organs (Bautista et al., 1990). In addition, LPS enhanced placental expression of 4-hydroxy-2-nonenal (HNE)-modified proteins, markers of oxidative stress (Ejima et al., 1999). Our earlier report showed that maternally administered LPS increased TBARS levels in fetal liver (Xu et al., 2005). A recent study indicated that *N*-acetylcysteine, a glutathione (GSH) precursor and direct antioxidant, protected against fetal death and preterm labor induced by maternal inflammation (Buhimschi et al., 2003). Ascorbic acid is an antioxidant and has been demonstrated to be effective on inhibiting LPS-evoked ROS production in lymphocytes (De la Fuente and Victor, 2001). A recent report showed that ascorbic acid decreased oxidative stress and prevented fetal malformation in experimental diabetic pregnancy (Cederberg et al., 2001). The present study found that pre-treatment with ascorbic acid reduced fetal mortality, alleviated LPS-induced IUGR, and reversed LPS-induced skeletal ossification retardation in caudal vertebrae, anterior and posterior phalanges, and supraoccipital bone. Furthermore, pre-treatment with ascorbic acid significantly attenuated LPS-induced increase in TBARS level in maternal liver, placenta and fetal liver. All these results suggest that ascorbic acid-mediated protection against LPS-induced IUFD and IUGR is, at least partially, associated with decreased lipid peroxidation.

The present study also investigated the effect of post-treatment with ascorbic acid on LPS-induced IUFD and IUGR. By contrast to pre-treatment, post-treatment with a single dose of ascorbic acid (500 mg/kg) showed a slight decrease in fetal mortality, although post-treatment reversed LPS-induced fetal growth development and skeletal ossification retardation. Furthermore, the protective effects of pre-treatment with ascorbic acid on LPS-induced IUFD were counteracted when the pregnant mice were administered with additional dose of ascorbic acid (500 mg/kg) at 3 h after LPS treatment. These results suggest that the effect of ascorbic acid depends on the schedule of administration. Pre-treatment with ascorbic acid protected against LPS-induced fetal death and reversed LPS-induced growth and skeletal development retardation, whereas post-treatment had less effect on LPS-induced IUFD.

Usually, ascorbic acid is mentioned as an “antioxidant”. However, under certain in vitro and in vivo conditions, ascorbic acid has been demonstrated to be also a prooxidant (Halliwell, 1996; Paolini et al., 1999; Otero et al., 1997). Previous study showed that treatment of cells with ascorbic acid 24 h prior to treatment of the cells with a radical generating system, this vitamin behaved as an antioxidant and protected against ROS-induced mutagenesis. Conversely, cotreatment of cells with ascorbic acid and the radical generating system, this vitamin behaved as a prooxidant and aggravated ROS-induced mutagenesis (Bijur et al., 1997). The present study also found that antioxidant or prooxidant activities of ascorbic acid greatly depend on the schedule of ascorbic acid administration and the dose administered. Pre-treatment with a single dose of ascorbic acid (500 mg/kg) significantly attenuated LPS-induced increase in TBARS levels in maternal liver, placenta and fetal liver, whereas post-treatment with a single dose of ascorbic acid (500 mg/kg) was less effective on LPS-induced lipid peroxidation. When the pregnant mice were administered additional dose of ascorbic acid (500 mg/kg) at 3 h after LPS treatment, this vitamin counteracted the effect of pre-treatment on LPS-induced lipid peroxidation in maternal liver and placenta, and eventually reduced the protective effects of pre-treatment on LPS-induced IUFD.

In summary, the present results suggest that the effects of ascorbic acid on LPS-induced IUFD and IUGR depend on the schedule of ascorbic acid administration. Pre-treatment with a single dose of ascorbic acid protected against LPS-induced fetal death and reversed LPS-induced growth and skeletal development retardation via counteracting LPS-induced oxidative stress. When administered at 3 h after LPS treatment, ascorbic acid had less effect on LPS-induced IUFD, although post-treatment significantly attenuated LPS-induced IUGR. Factually, when administered additional dose of ascorbic acid at 3 h after LPS treatment, this “antiox-

idant" reduced the protective effects of pre-treatment with ascorbic acid on LPS-induced IUFD.

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