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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