Molecular and Cellular Endocrinology 423 (2016) 51–59

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Rosiglitazone pretreatment protects against lipopolysaccharide-induced fetal demise through inhibiting placental inflammation

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ARTICLE INFO

Article history:
Received 30 November 2015
Received in revised form
25 December 2015
Accepted 6 January 2016
Available online 8 January 2016

Keywords:
Rosiglitazone
Peroxisome proliferator-activated receptor-γ
Lipopolysaccharide
Inflammation
Fetal death

ABSTRACT

Peroxisome proliferator-activated receptor (PPAR)-γ is highly expressed in human and rodent placentas. Nevertheless, its function remains obscure. The present study investigated the effects of rosiglitazone, a PPAR-γ agonist, on LPS-induced fetal death. All pregnant mice except controls were intraperitoneally injected with LPS (150 μg/kg) daily from gestational day (GD)15 to GD17. As expected, maternal LPS injection caused placental inflammation and resulted in 63.6% fetal death in dams that completed the pregnancy. Interestingly, LPS-induced fetal mortality was reduced to 16.0% when pregnant mice were pretreated with RSG. Additional experiment showed that rosiglitazone pretreatment inhibited LPS-induced expressions of tumor necrosis factor (*Tnf*)-α, interleukin (*Il*)-1β, *Il-6*, macrophage inflammatory protein (*Mip*)-2 and keratinocyte-derived chemokine (*Kc*) in mouse placenta. Although rosiglitazone had little effect on LPS-evoked elevation of IL-10 in amniotic fluid, it alleviated LPS-evoked release of TNF-α and MIP-2 in amniotic fluid. Further analysis showed that pretreatment with rosiglitazone, which activated placental PPAR-γ signaling, simultaneously suppressed LPS-evoked nuclear factor kappa B (NF-κB) activation and blocked nuclear translocation of NF-κB p65 and p50 subunits in trophoblast giant cells of the labyrinth layer. These results provide a mechanistic explanation for PPAR-γ-mediated anti-inflammatory activity in the placentas. Overall, the present study provides additional evidence for roles of PPAR-γ as an important regulator of placental inflammation.

Lipopolysaccharide (LPS) is a toxic component of cell walls in gram-negative bacteria and is widely present in the digestive tracts of humans and animals (Jacob et al., 1997). Humans are constantly exposed to low levels of LPS through infection. Gastrointestinal inflammatory diseases and excess alcohol intake are known to increase permeability of LPS from gastrointestinal tract into blood (Zhou et al., 2003). LPS has been associated with adverse developmental outcomes. According to several earlier reports, maternal LPS exposure at early gestational stage induced embryonic resorptions and fetal death in rodent animals (Gendron et al., 1990; Ogando et al., 2003). Moreover, maternal LPS exposure at middle gestational stages caused fetal death and abortion (Leazer et al., 2002). Several studies demonstrated that maternal LPS exposure during organogenesis induced fetal malformations and skeletal abnormalities in rats, golden hamsters and mice (Ornoy and Altshuler, 1976; Lanning et al., 1983; Collins et al., 1994; Zhao et al., 2008, 2014; Fu et al., 2014; Chen et al., 2015a). Increasing evidence demonstrated that maternal LPS exposure at late gestational stage caused preterm delivery, fetal death, fetal growth restriction and skeletal development retardation (Rivera et al., 1998; Buhimschi et al., 2003; Xu et al., 2005, 2006a, 2007a; Guo et al., 2013). Moreover, maternal LPS exposure during pregnancy caused age- and gender-dependent impairments of neurobehavioral development in offspring (Romero et al., 2007; Wang et al., 2010; Xia et al., 2014; Solati et al., 2015; Zager et al., 2015). In addition, maternal LPS exposure at late gestational stage permanently impaired steroidogenesis and spermatogenesis in male offspring (Wang et al., 2014).

Numerous studies demonstrate that inflammatory cytokines

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http://dx.doi.org/10.1016/j.mce.2016.01.004
0303-7207/

and chemokines, such as tumor necrosis factor alpha (TNF-α) and interleukin (IL)-8, are associated with adverse pregnant outcomes including preterm delivery, fetal death and fetal growth restriction (Leazer et al., 2002; Xu et al., 2006b; Rode et al., 2012; Cotechini et al., 2014). Indeed, maternal LPS exposure elevated the levels of inflammatory cytokines in maternal serum, amniotic fluid, fetal liver and fetal brain (Xu et al., 2007b; Ning et al., 2008; Li et al., 2008). Several studies found that some chemicals alleviated LPS-induced abortion, preterm birth, fetal death, neural tube defects and fetal growth restriction through their anti-inflammatory activity (Chen et al., 2006, 2012; Awad et al., 2011; Zhao et al., 2013; Zhao et al., 2014; Fu et al., 2014; Chen et al., 2015b).

Peroxisome proliferator-activated receptor (PPAR)-γ is a nuclear receptor with an immune modulation and antiinflammatory activity (Ahmadian et al., 2013). An early report showed that rosiglitazone (RSG), an agonist of PPAR-γ, inhibited the release of inflammatory cytokines during LPS-evoked acute kidney injury (Lee et al., 2005). An in vitro study found that RSG inhibited LPS-induced up-regulation of inflammatory cytokines in HK-2 cells (Wang et al., 2011a). Indeed, PPAR-γ is highly expressed in human and rodent placentas (Wang et al., 2002; Capobianco et al., 2013). In the present study, we investigated the effects of RSG pretreatment on LPS-induced fetal death. We demonstrated that pretreatment with RSG protected mice from LPS-induced fetal death through inhibiting placental inflammation. Our results provide additional evidence for roles of PPAR-γ as an important regulator of placental inflammation.

1. Materials and methods

1.1. Chemicals and reagents

Lipopolysaccharide (*Escherichia coli* LPS, serotype 0127:B8) and RSG were purchased from Sigma Chemical Co. (St. Louis, MO). Nuclear factor-kappa B p65 (NF-κB p65) and Lamin antibodies were from Santa Cruz Biotechnologies (Santa Cruz, CA). NF-κB p50 antibodies were from Cell Signaling Technology (Beverley, MA). PPAR-γ, Phosphor-inhibitor of kappa B (p-IκB) and IκB antibodies were purchased from Abcam (Cambridge, MA). Chemiluminescence (ECL) detection kit was from Pierce Biotechnology (Rockford, IL). TRI reagent was from Molecular Research Center, Inc (Cincinnati, Ohio). RNase-free DNase was from Promega Corporation (Madison, WI). All the other reagents were from Sigma or as indicated in the specified methods.

1.2. Animals and treatments

The ICR 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 (GD) 0. The present study consisted of two separate experiments.

1.2.1. Experiment 1

To investigate the effects of RSG on LPS-induced fetal demise, forty pregnant mice were divided into four groups randomly. In the RSG alone group and the RSG + LPS group, pregnant mice were orally administered with RSG (10 mg/kg) daily from GD13 to GD17. The dose of RSG used in the present study referred to others (Hwang et al., 2012). In the LPS alone group and the RSG + LPS group, pregnant mice were intraperitoneally (i.p.) injected with LPS (150 μg/kg) daily from GD15 to GD17. The dose of LPS used in the present study referred to others with minor modulation (Xu et al., 2007a). In control group, pregnant mice were i.p. injected with saline daily from GD15 to GD17. All dams were sacrificed 24 h after the last LPS injection (on GD18). For each litter, the number of live fetuses, dead fetuses and resorption sites were counted. Live fetuses were weighed and crown-rump lengths were measured in control group and RSG alone group.

1.2.2. Experiment 2

To investigate the effects of RSG on LPS-induced placental inflammatory signaling, twenty-four pregnant mice were divided into four groups randomly. In the RSG alone group and the LPS + RSG group, pregnant mice were orally administered with RSG (10 mg/kg) daily from GD13 to GD15. In the LPS alone group and the RSG + LPS group, pregnant mice were i.p. injected with LPS (150 μg/kg) on GD15. In control group, pregnant mice were i.p. injected with saline on GD15. Preliminary experiment showed that the expressions of most inflammatory cytokines and chemokines in the placentas were highest at 1.5 h after LPS injection. Thus, all dams were sacrificed at 1.5 h after LPS injection. Maternal serum and amniotic fluid were collected for measurement of TNF-α, MIP-2 and IL-10. The placentas were collected for real-time RT-PCR and immunoblots. Some placentas were collected for histopathology and immunohistochemistry. 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.

1.3. Isolation of total RNA and real-time RT-PCR

Total RNA was extracted using TRI reagent. RNase-free DNase-treated total RNA (1.0 μg) was reverse-transcribed with AMV (Pregmega). Real-time RT-PCR was performed with a LightCycler 480 SYBR Green I kit (Roche Diagnostics GmbH) using gene-specific primers as listed in Table 1. The amplification reactions were carried out on a LightCycler 480 Instrument (Roche Diagnostics GmbH) with an initial hold step (95 °C for 5 min) and 50 cycles of a three-step PCR (95 °C for 15 s, 60 °C for 15 s, 72 °C for 30 s).

1.4. Immunoblots

For nuclear protein extraction, total lysate from the placental tissues was suspended in hypotonic buffer (50 mM Tris–HCl,

Table 1
Oligonucleotide sequences and size of primers.

Genes	Sequences	Sizes (bp)
18S	Forward: 5′- GTAACCCGTTGAACCCCATT-3′ Reverse: 5′- CCATCCAATCGGTAGTAGCG-3′	151
Ppar-γ	Forward: 5′- GGGCTGAGGAGAAGTCACAC-3′ Reverse: 5′- TCAGTGGTTCACCGCTTCTT-3′	144
Tnf-α	Forward: 5′- CCCTCCTGGCCAACGGCATG -3′ Reverse: 5′- TCGGGGCAGCCTTGTCCCTT -3′	109
Il-1β	Forward: 5′- GCCTCGTGCTGTCGGACCCATAT-3′ Reverse: 5′- TCCTTTGAGGCCCAAGGCCACA -3′	143
Il-6	Forward: 5′- AGACAAAGCCAGAGTCCTTCAGAGA -3′ Reverse: 5′- GCCACTCCTTCTGTGACTCCAGC -3′	146
Kc	Forward: 5′-ACTCAAGAATGGTCGCGAGG-3′ Reverse: 5′-GTGCCATCAGAGCAGTCTGT-3′	123
Mip-2	Forward: 5′- TTGCCTTGACCCTGAAGCCCCC -3′ Reverse: 5′- GGCACATCAGGTACGATCCAGGC -3′	175