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Curcumin improves LPS-induced preeclampsia-like phenotype in rat by inhibiting the TLR4 signaling pathway

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ABSTRACT

Introduction: Abnormal inflammation mediated by Toll-like receptor 4 (TLR4) signaling pathway contributes to preeclampsia (PE). Because curcumin can inhibit TLR4 signaling pathway, we investigated its effects on a PE rat model.
Methods: Twenty-one pregnant rats were randomly divided into three groups: 1) seven rats were injected 0.5 μg/kg lipopolysaccharide (LPS) on gestational day (GD) 5 to create a PE model (LPS-treated group), 2) seven rats were injected with a similar dosage of LPS and further treated with curcumin (0.36 mg/kg) (LPS-curcumin-treated group), 3) seven rats received saline (control group). Blood pressure and urinary protein level were observed. Immunostaining and periodic acid-Schiff staining of placenta were conducted. TLR4 and downstream Nuclear Factor-κB (NF-κB) expressions of placenta were analyzed by Western blot and immunohistochemistry. IL-6 and MCP-1 in rat serum and placenta were determined by ELISA and qRT-PCR.
Results: Compared to LPS-treated group, LPS-curcumin-treated group had decreased blood pressure and urinary protein level, similar to control group. Furthermore, deficient trophoblast invasion and spiral artery remodeling induced by LPS were improved by curcumin. Increased TLR4, NF-κB and IL-6, MCP-1 protein expressions in LPS-treated group were significantly decreased after curcumin administration.
Discussion: Curcumin improves the PE-like phenotype in rat model by reducing abnormal inflammation related to TLR4 signaling pathway.

1. Introduction

Preeclampsia (PE) affects 2–8% of all pregnancies worldwide and remains a leading cause of maternal and fetal mortality and morbidity [1,2]. Although the etiology remains elusive, accumulating evidence has shown it is associated with an aberrant maternal-fetal inflammatory response, which induces inadequate spiral artery (SA) remodeling, an important alteration in the pathophysiology of PE [3,4].

Studies of term placenta show that anomalies of Toll-like receptor 4 (TLR4) signaling pathway are involved in immune maladaptation and abnormal placentation of PE [5–7]. TLR4, expressed in trophoblast in the placenta [8], has been established as a major family of pattern recognition receptors (PRRs) [5]. In addition to endogenous ligands (i.e. heat shock protein 60 (HSP60)), lipopolysaccharide (LPS) is a major exogenous ligand recognized by TLR4. Activation of TLR4 pathway triggers effective molecules associated with PE, including monocyte chemoattractant protein-1 (MCP-1) and interleukin (IL)-6 [9,10]. In 1994, Faas et al. developed a classical animal model for PE by injecting LPS into pregnant rat on gestational day (GD) 14 [11]. Recently, we also have generated a similar model by intravenous administration LPS on GD 5. Our results indicate that abnormal activation of TLR4 may lead to excess inflammation and contribute to a PE-related phenotype [12].

Curcumin (Fig. 1) is used as a food coloring agent as well as a therapeutic drug in traditional medicine [13,14], which is considered safe [15,16]. It has been reported that curcumin can target to TLR4 and downstream Nuclear Factor-κB (NF-κB) and activated

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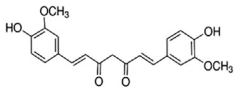

Fig. 1. Chemical structure of curcumin.

protein-1 (AP-1) [17,18]. Extensive studies have shown curcumin has an ability to treat diabetic nephropathy, traumatic brain injury and periodontal disease by down-regulating TLR4 signaling pathway [19–21]. The latest study has revealed curcumin exerts an anti-inflammatory role in preventing pregnant women from preterm birth [22].

Therefore, this study explored whether curcumin could improve PE-like phenotype in LPS-induced rat model through inhibition of overactive TLR4 signaling pathway.

2. Materials and methods

2.1. Animals and experimental protocol

All procedures and protocols were approved by the Committee on the Ethics of Animal Experiments of Drum Tower Hospital (SYXK 2009-0017) and all animal research were consistent with the guidelines of Experimental Animals Management Committee (Jiangsu Province, China).

Female Sprague-Dawley rats (10 weeks old) were housed in a humidity- and light-controlled room. They were overnight mated with strong males at a 1:1 ratio. Vaginal spermatozoa were used as a marker to determine the success of maternal pregnancy and the day was recorded as GD 0. Pregnant rats were randomly divided into three groups: control group (N = 7); LPS-treated group (N = 7); LPS-curcumin-treated group (N = 7). Rats in control group were treated with 2 mL of saline. For LPS-treated group, the treatment (0.5 μg/kg LPS (*Escherichia coli* serotype 0111:B4, Sigma-Aldrich), dissolved in 2 mL saline) was injected on GD 5 [12]. To determine the curcumin dose, we investigated its suppression on LPS-induced activation of TLR4 signaling pathway in human-trophoblast-derived HTR-8/SVneo cells. We evaluated the inhibition of TLR4 signaling pathway at five different curcumin concentrations (0, 6.25, 12.5, 25 and 50 μM) and determined 12.5 μM (approximate 0.36 mg/kg) as the intervention dose [Supplementary Fig. 1]. Curcumin (Sigma C-1356) (dissolved in dimethyl sulfoxide (DMSO) and suspended in 1 mL saline) was injected 20 mins after LPS administration. All injections were conducted through the tail vein [12]. On GD 18, pregnant rats were anesthetized by 10% chloral hydrate (3 mL/kg, intraperitoneally). Afterwards, we obtained venous specimens for routine blood and cytokine examination. The numbers of viable and resorbed pups were recorded. The wet weights of pups and placentas were measured.

2.2. Blood pressure and urinary protein level

The rats' systolic blood pressure (SBP) (08:00 a.m.–10:00 a.m.) were monitored every 3 days by tail-cuff plethysmography (BP-98A; Softron, Tokyo, Japan). Briefly, each rat was warmed to 38 °C and SBP was assessed continuously 15 times, in which 3 continuous values of variation of less than 6 mmHg were averaged to define maternal SBP.

The rat urine (20:00 p.m.–10:00 a.m.) was collected every 3 days with dams housed individually in metabolic cages without food. Urinary protein level was measured using the pyrogallol red method [23], and urinary creatinine concentrations were determined using a Creatinine Assay Kit (Jiancheng Bioengineering Institute, China).

2.3. Histology and immunostaining

Placenta and kidney tissue samples were fixed in 10% formaldehyde, dehydrated in a series of graded alcohols and embedded in paraffin. Kidney slices were stained with hematoxylin-eosin (HE) and periodic acid-Schiff (PAS). Implantation site (placenta + mesometrial triangle (MT)) were cut in at least 3 serial sections. For each implantation site, select sections containing a central maternal arterial channel (Fig. 3A) were stained with PAS (a fibrinoid tissue marker), cytokeratin (1:500 dilutions, ProteinTech, Chicago, USA) (a trophoblast marker), and α-actin (1:800 dilutions, Abcam) (a vascular smooth muscle cells (VSMCs) marker) [24,25]. Image J analysis system was used to assess trophoblast invasion and SA remodeling. Briefly, the lumen of each SA cross section was manually delineated and stretches of trophoblast, fibrinoid, and VSMCs were traced separately over the lumen contour tracing. The percentages of cytokeratin, fibrinoid, and α-actin staining of the corresponding SA contour were calculated. TLR4 and p65 expressions in rat placenta were determined using corresponding antibodies (TLR4, 1:100 dilutions, Abcam; p65, 1:200 dilutions, Bioworld Technology) [12].

2.4. RNA isolation and quantitative real-time PCR

Total RNA was extracted using TRIzol reagent (Invitrogen). cDNA was synthesized from 1 μg of purified RNA using a Prime Script RT Master Mix kit (Takara) according to the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) was performed [26] and the specific primers used for PCR analysis were listed in Table 1. Data were analyzed by the $2^{-\Delta\Delta CT}$ method, and GAPDH was used for normalization.

2.5. Enzyme-linked immunosorbent assay

Cytokines IL-6 and MCP-1 in rat serum were quantitatively detected by enzyme-linked immunosorbent assay (ELISA) kits (rat IL-6 (detection range, 2.57–8000 pg/mL)(Intra-Assay CV%: <10%, Inter-Assay CV%: <12%): Sunny ELISA Kits, MultiSciences, China; rat MCP-1 (detection range, 4.7–500 pg/mL)(Intra-Assay CV%: <10%, Inter-Assay CV%: <12%): eBioscience) according to the manufacturer's instructions.

2.6. Western blot analysis

Proteins were prepared and separated by SDS-PAGE [27]. Immunoblotting was performed with primary antibodies raised against TLR4 (1:1000 dilutions; Santa Cruz), NF-κB p65 (1:1000 dilutions; Santa Cruz), Jun-B (1:1000 dilutions; Santa Cruz) and GAPDH (1:5000 dilutions; Bioworld). Immunodetection was accomplished using a goat anti-rabbit (1:5000 dilutions; Bio-Rad Laboratories) or rabbit anti-mouse (1:10000; Bio-Rad Laboratories) HRP conjugated secondary antibody, and an enhanced chemiluminescence detection kit (Millipore).

Table 1
Oligonucleotide primer sequences for quantitative real-time PCR.

Species	Genes	Forward primer (5′→3′)	Reverse primer (5′→3′)
Rat	IL-6	GCCCTTCAGGAACAGCTATG	CAGAATTGCCATTGCACAAC
Rat	MCP-1	TTCACAGTTGCTGCCTGTAG	TCTGATCTCACTTGGTTCTGG
Rat	GAPDH	ATGGGAAGCTGGTCATCAAC	GGATGCAGGGATGATGTTCT