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Effect of curcumin on expressions of NF-κBp65, TNF-α and IL-8 in placental tissue of premature birth of infected mice

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ABSTRACT

Objective: To observe the effect of curcumin on expressions of nuclear transcription factor-kappa Bp65 (NF-κBp65), TNF-α and IL-8 in placental tissue of premature birth of infected mice induced by lipopolysaccharide (LPS).

Methods: A total of 60 C57BL/6 mice pregnant with 15 d were collected and randomly divided into control group, model group, treatment group and preventative group. LPS was repeatedly injected in abdominal cavity to construct infected premature birth model, while mice of control group were given with 100 mg/kg of vitamin C through abdominal cavity injection and mice of treatment group and preventative group were given curcumin of 100 mg/kg through abdominal cavity injection after modeling operation and before 1 d of modeling operation, respectively. A total of 5 mice of four groups respectively were executed by cervical dislocation after 6 h, 12 h and 24 h after constructing model. Placental tissues were collected and the immunohistochemical method SABC of immunologic tissue was used to detect the expression of NF-κBp65, TNF-α and IL-8 and peripheral blood of executed mice after 24 h was collected to detect the concentrations of IL-8, malondialdehyde (MDA) and superoxide dismutase (SOD), meanwhile live birth rate of four groups was contrasted.

Results: Staining intensity of NF-κBp65, TNF-α and IL-8 in placental tissue of treatment group and preventative group was significantly higher than control group but lower than model group ($P < 0.05$). Level of serum IL-8 and MDA of control group was significantly lower than the other three groups ($P < 0.05$) and level of blood of SOD in model group was significantly lower than control group ($P < 0.05$). Levels of serum IL-8 and MDA of treatment group and preventative group were significantly lower than model group ($P < 0.05$) while level of SOD was significantly higher than model group ($P < 0.05$). Live birth rate of treatment group and preventative group was significantly higher than model group ($P < 0.05$).

Conclusions: Curcumin can effectively prevent the active pathway of NF-κB in pregnant tissue of premature birth of infected mice, reduce the expression of TNF-α and IL-8 and relieve the damage of lipid peroxide of oxidative stress of LPS on mother-fetus and further to achieve the objective of preventing and curing premature birth induced with infection.

1. Introduction

Premature birth refers to pregnancy and delivery less than 37 weeks which is an important reason to cause increase of morbidity and mortality rate of perinatal infants. The incidence of premature birth accounts for 5%–15% of common pregnancy complication induced by various reasons [1–3]. Some researchers believed that [4,5] a variety of pathogenic microorganisms were associated with the morbidity of premature birth and that infection, which was mostly presented by subclinical infection is an important factor to induce premature birth and manifestations. In recent years, a plenty of studies were verified that [6–9] cytokines are closely associated with the occurrence of premature birth, thus the regulation of expression of cytokines NF-κB has attached great attention in clinic. NF-κB is an essential factor for highly transcribing proinflammatory gene and can efficiently induce the genetic

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expression of adhesion molecule, chemotactic factor, many cytokines and acute phase protein. The active pathway and nuclear translocation of NF-κB can cause increase in the synthesis of inflammatory cytokines and is the key reason to induce infected premature birth [10,11]. Some studies have reported that [12], curcumin has the effect to down-regulate the inflammatory reaction of *in vitro* pregnant tissue and can significantly lower the intrauterine death rate of animal fetal rats which has certain prospects on preventing infected premature birth. This study aims to observe the control efficiency and action mechanism of curcumin on the treatment of premature birth model of infected mice induced by lipopolysaccharide (LPS) and to construct the premature birth model of C57BL/6 mice which were given curcumin to prevent and treat and explore its action mechanism in order to provide theoretical basis for clinical application.

2. Methods and materials

2.1. Experimental animals

A total of 60 C57BL/6 mice pregnant with 15 d which were weighed 40–50 g were collected and provided by Center of Animal Experimental, Shaanxi University of Traditional Chinese Medicine. All mice were cleaned and fed and fed with water freely and were lighted for 12 h day and night. This study was accomplished in experimental center of Shaanxi University of Traditional Chinese Medicine and the disposition of experimental animals complied with related regulations of Regulations for the Administration of Affairs Concerning Experimental Animals and was obtained the approval by Ethics Committee of Shaanxi University of Traditional Chinese Medicine.

2.2. Instruments and reagents

Microsurgical instrument was obtained from Shanghai Instrument Factory Co., Ltd.; CM2027 Constant Freezing Microtome was obtained from Leica, Germany; micropipettor was obtained from Lab Mate; pH instrument was obtained from Hilton, USA; optical microscopy system was obtained from Olympus, Japan; Heto Holten Denmark and Decolorization Shaker were obtained from Shanghai Instrument Factory Co., Ltd.; micro adding sample appliance was obtained from Glison, France; Mig2000 model image analyser and Mig2000 image analysis software were obtained from Chongqing University of China. Curcumin (batch No.: Z600725) and LPS (batch No.: MFCD00164401) were obtained from Sigma Co., Ltd. of USA; FOTODYNEGel Document System was obtained from PE Co., Ltd. of USA; SABC immunohistochemical kit and DAB chromogenic reagent kit were obtained from Zhongshan Co., Ltd. of Beijing and rat monoclonal antibodies of TNF-α, IL-8, NF-κ and B p65 were obtained from Wuhan Boster Biotech Co. Ltd.

2.3. Groups and treatment

A total of 60 mice were randomly divided into control group, model group, treatment group and preventative group with 15 mice in each. To construct infected premature birth model, mice of model group, treatment group and preventative group were repeatedly injected with LPS in abdominal cavity and methods were the following: LPS was configured into 0.01 mg/mL, 0.1 mg/mL and 1 mg/mL and 0.1 mL of LPS of above concentrations was injected into mice of model group, treatment group and preventative group by intraperitoneal injection, while mice of control group were given with 100 mg/kg of vitamin C through abdominal cavity injection. About 100 mg/kg curcumin was given into mice of treatment group by intraperitoneal injection after modeling operation and 100 mg/kg curcumin were given into mice of preventative group through abdominal cavity injection before 1 d of modeling operation for preventive treatment.

2.4. Observation items

There were four groups of 15 mice for each of which every 5 mice were executed by cervical dislocation after 6 h, 12 h and 24 h of constructing model. Placental tissue was collected and the immunohistochemical method SABC was used to detect the expression of nuclear transcription factor-kappa Bp65 (NF-κBp65), TNF-α and IL-8 and the images collected from microscope were entered into Mig2000 image analysis software, meanwhile semi-quantitative analysis was conducted on the dyeing NF-κBp65, TNF-α and IL-8 of immunohistochemical method. Peripheral blood of 5 executed mice after 24 h of four groups was collected and serum was separated. ELISA method was used to detect the concentrations of IL-8, malondialdehyde (MDA) and superoxide dismutase (SOD), meanwhile live birth rate of four groups was contrasted. The computing method of live birth rate: placental tissue of sacrificed mice was collected by touching fetal rats using swab and if limbs were responding for the touchness, it was judged as live birth.

2.5. Statistical methods

Measurement data were expressed by mean ± SD. Mean value was inspected by *t*-text and live birth rate was compared by Chi-square test. Data obtained from this study were inputted and processed by Microsoft EXCEL2000 software package. $P < 0.05$ was considered statistically significant in the compared data among groups.

3. Results

3.1. Comparison of staining intensity of NF-κBp65, TNF-α and IL-8 in placental tissue of four groups

Staining intensity of NF-κBp65, TNF-α and IL-8 in placental tissue of model group was significantly higher than those in control group, treatment group and preventative group ($P < 0.05$) and staining intensity of NF-κBp65, TNF-α and IL-8 in placental tissue of treatment group and preventative group was significantly higher than those in control group ($P < 0.05$) (Table 1).

3.2. Comparison of serum IL-8, MDA and SOD levels of executed mice after 24 h in four groups

Level of serum IL-8 and MDA of model group, treatment group and preventative group was significantly higher than those

Table 1
Comparison of staining intensity of NF-κBp65, TNF-α and IL-8 in placental tissue of four groups [n = 15; OD value (%)] 6, 12 and 24 h after model establishment.

Group	NF-κBp65			TNF-α			IL-8		
	6	12	24	6	12	24	6	12	24
Control group	13.0 ± 1.2	13.4 ± 1.6	16.5 ± 2.2	7.1 ± 1.3	7.0 ± 1.6	16.4 ± 1.3	12.0 ± 0.9	12.4 ± 1.3	18.9 ± 1.0
Model group	15.2 ± 0.8*	21.2 ± 1.6*	24.5 ± 1.7*	8.0 ± 1.7*	14.1 ± 0.9*	31.4 ± 1.4*	17.1 ± 1.3*	24.0 ± 0.8*	27.6 ± 1.4*
Treatment group	13.9 ± 1.3$^{*\#}$	14.5 ± 1.1$^{*\#}$	18.1 ± 1.5$^{*\#}$	8.0 ± 1.5$^{*\#}$	8.7 ± 1.2$^{*\#}$	19.9 ± 1.8$^{*\#}$	13.1 ± 0.9$^{*\#}$	13.8 ± 1.3$^{*\#}$	25.4 ± 1.4$^{*\#}$
Preventative group	13.8 ± 1.2$^{*\#}$	13.5 ± 1.8$^{*\#}$	17.1 ± 2.1$^{*\#}$	7.9 ± 1.5$^{*\#}$	8.0 ± 1.3$^{*\#}$	17.8 ± 1.8$^{*\#}$	12.9 ± 1.0$^{*\#}$	13.0 ± 1.5$^{*\#}$	24.8 ± 1.4$^{*\#}$

Five mice were executed by cervical dislocation after 6, 12, and 24 h of constructing model. $^{*}P < 0.05$ compared with control group; $^{\#}P < 0.05$ compared with model group.

in control group ($P < 0.05$) and level of blood of SOD in model group was significantly lower than control group ($P < 0.05$). Level of blood of SOD in treatment group and preventative group was significantly higher than control group ($P < 0.05$). Levels of serum IL-8 and MDA of treatment group and preventative group were significantly lower than model group ($P < 0.05$) while level of SOD was significantly higher than model group ($P < 0.05$) (Table 2).

Table 2
Comparison of serum IL-8, MDA and SOD levels of executed mice after 24 h in four groups (n = 5).

Group	IL8 (pg/mL)	MDA (nmol/mL)	SOD (U/mL)
Control group	210.2 ± 9.0	0.4 ± 0.2	12.5 ± 2.9
Model group	900.7 ± 23.3*	2.7 ± 0.4*	11.7 ± 2.2*
Treatment group	560.3 ± 15.8$^{*\#}$	1.9 ± 0.6$^{*\#}$	25.6 ± 3.6$^{*\#}$
Preventative group	420.7 ± 12.0$^{*\#\triangle}$	0.8 ± 0.7$^{*\#\triangle}$	45.0 ± 2.4$^{*\#\triangle}$

$^{*}P < 0.05$ compared with control group; $^{\#}P < 0.05$ compared with model group; $^{\triangle}P < 0.05$ compared with treatment group.

3.3. Comparison of live birth rate of mice in four groups

The live birth rates of control group, model group, treatment group and preventative group were 96.7% (58/60), 4.8% (3/62), 68.3% (41/60) and 84.6% (55/65), respectively. Live birth rate of model group, treatment group and preventative group was all lower than control group ($P < 0.05$), while live birth rate of treatment group and preventative group was significantly higher than model group ($P < 0.05$).

4. Discussion

According to statistics [13,14], the incidence of premature birth every year accounts for about 5%–18% of the overall pregnancy worldwide and about one million of premature neonates die of related complications of premature birth each year. Premature birth is the important factor to cause illness and death of perinatal infant. Premature breaking of fetal membrane, excessive swell of uterus, lower genital tract infection and complications of pregnancy are high risk factors inducing premature birth. Nevertheless, in recent years, many studies have verified that whether there is an infection, inflammatory reaction and its morbidity can play a vital role in the occurrence of premature birth [15–17].

Premature birth is a process with participation of multiple systems. In the occurrence of premature birth, various inflammatory cells and lymphocytes gather in maternal–fetal interface and produce a large number of cytokines and chemokines which increase levels of prostaglandin and protease and cause uterine contraction and premature rupture of fetal membranes [18]. While NF-κB is an important infectious regulatory factor in human body which exists widely in cells and mainly presents by p50/p65 heterodimer. After NF-κB transfers into the nuclear, it can combine with NF-κB of promoter of targeted gene to induce the transcription of many cytokines [19]. Some studies have found that [20], NF-κB also has role to regulate inflammatory signaling pathways and a variety of chemokines and its abnormal high expression in pregnant tissue can cause a large number of secretion of inflammatory factor in body, thus further participate into the morbidity of premature birth. The results of this study showed that staining intensity of NF-κBp65 in placental tissue of model group was significantly higher than control group, treatment group and preventative group ($P < 0.05$) and staining intensity of NF-κBp65 in placental tissue of treatment group and preventative group was significantly higher than control group ($P < 0.05$) and also verified that the high expression state of NF-κBp65 protein in placental tissue of model group mice is closely related with the morbidity of premature birth. However, because curcumin was used to prevent and treat this disease in treatment group and preventative group, the expression of NF-κBp65 protein in placental tissue was lower than model group which indicated that curcumin can down-regulate the expression of NF-κBp65 protein in placental tissue of premature birth model and achieve the objective of preventing and treating infected premature birth through this mechanism.

Curcumin is an extractive of traditional Chinese medicine, *Carcuma longa* has extensive pharmacological effects. Several studies have confirmed that the toxicity of curcumin is very low and it has significantly effects such as antioxidant, anti-inflammatory, suppressing tumor growth, inhibition of angiogenesis and reducing blood fat [12]. Some experiments reported [21–23] that curcumin can inhibit the expression of NF-κBp65 and TNF-α protein in placental tissue and further to reduce inflammatory reaction in body and also achieve the effect of preventing and controlling the occurrence of premature birth. Some studies have shown that [24,25] LPS can stimulate TNF-α and IL-8 in pregnant tissue, the production of prostaglandin, induce the release of active oxygen, cause unbalance of oxidative stress in maternal–fetal interface, make environmental degradation of uterus and lead to dead fetus in the uterus and there is no special effect of controlling measure so far. The results of this study showed that staining intensity of NF-κBp65, TNF-α and IL-8 in placental tissue of model group was significantly higher than those in control group, treatment group and preventative group ($P < 0.05$) and staining intensity of NF-κBp65, TNF-α and IL-8 in placental tissue of treatment group and preventative group was significantly higher than those in control group ($P < 0.05$) which indicated that curcumin

possesses role to down-regulate the expression of NF-κBp65, TNF-α and IL-8 and lower inflammatory reaction of body. An experiment have confirmed that curcumin can directly remove free radicals in the body (ROS and RNS) and has more significant effect on removing ROS compared with other polyphenol compounds. In addition, curcumin also has significant effect on inhibiting lipid peroxidation and maintaining various antioxidant enzyme activities. Lipid peroxidation is a chain reaction mediated by free radical and curcumin inhibiting lipid peroxidation is mainly due to it can remove those active radicals which participate in the peroxidatic reaction.

In the study, level of serum IL-8 and MDA of model group, treatment group and preventative group was significantly higher than those in control group ($P < 0.05$) and level of blood of SOD in model group was significantly lower than control group ($P < 0.05$) while level of blood of SOD in treatment group and preventative group was significantly higher than control group ($P < 0.05$). Levels of serum IL-8 and MDA of treatment group and preventative group were significantly lower than model group ($P < 0.05$) while level of SOD was significantly higher than model group ($P < 0.05$) and the incubation periods of childbirth in treatment group and preventative group were significantly higher than control group ($P < 0.05$). Live birth rate of model group, treatment group and preventative group was significantly higher than control group ($P < 0.05$), which indicated that curcumin can effectively prevent the active pathway of NF-κB in premature birth of infected mice, reduce the expression of TNF-α and IL-8 and relieve the damage of lipid peroxide of oxidative stress of LPS on mother-fetus and effectively to prevent and cure premature birth induced with infection.

The results of this study indicated that curcumin can reduce inflammatory reaction of mice and relieve the damage of lipid peroxide of oxidative stress of LPS on mother-fetus through preventing the active pathway of NF-κB in pregnant tissue of premature birth of infected mice to effectively prevent and control premature birth induced with infection.

Conflict of interest statement

We declare that we have no conflict of interest.

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