EXPERIMENTAL RESEARCH

Role of Shenfu Injection (参附注射液) in Rats with Systemic Inflammatory Response Syndrome*

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ABSTRACT **Objective:** To investigate the role of Shenfu Injection (参附注射液, SFI) in rats with systemic inflammatory response syndrome (SIRS). **Methods:** The SIRS rat model was induced by the intravenous injection of lipopolysaccharide (LPS). Forty-five male Wistar rats were randomly divided into 3 groups, the sham operative control group (control group, *n*=5), the SIRS model group (model group, *n*=20) and the SFI treatment group (SFI group, *n*=20). LPS was injected through the external jugular vein (12 mg/kg, 6 mg/mL) to all rats except for those in the control group, and SFI (10 mL/kg) was given to those in the SF group only once through intraperitoneal injection, while the normal saline (10 mL/kg) was given to those in the model group. For those in the control group, normal saline was given through the external jugular vein (2 mL/kg) and intraperitoneal injection (10 mL/kg). Then, rats in the model group and SFI group were divided into 4 subgroups according to the time points, i.e., 1 h, 2 h, 4 h and 6 h subgroups, 5 rats in each group. The activity of nuclear factor of κB (NF-κB) of in blood mononuclear cells and the plasma levels of tumor necrosis factor-α (TNF-α) and interleukin 6-(IL-6) were determined using enzyme-linked immunoabsorbent assay (ELISA) at 1 h, 2 h, 4 h and 6 h after modeling. Histopathologic changes of the lung and liver were observed under a light microscope. **Results:** Compared with the control group, the activity of NF-κB in mononuclear cells and the plasma level of TNF-α were obviously increased at each time points (all $P<0.01$), reaching the peaks at 2 h after modeling. The plasma level of IL-6 increased gradually as time went by in the model group ($P<0.01$). Pathological examination showed pulmonary alveoli hemorrhage, edema and inflammatory cell infiltration in the lung tissue, and angiotelectasis, congestion, and local necrosis in the liver tissue in the model group. Compared with the model group, the activity of NF-κB and the levels of TNF-α and IL-6 in plasma decreased significantly in the SFI group ($P<0.01$), and the pathological injury in the lungs and liver was significantly alleviated. **Conclusion:** SFI plays a protective role by inhibiting the activity of NF-κB, and reducing the expressions of TNF-α and IL-6 in SIRS rats.

KEY WORDS Shenfu Injection, systemic inflammatory response syndrome, mononuclear cells, lipopolysaccharide, nuclear factor-κB, tumor necrosis factor-α, interleukin-6

Systemic inflammatory response syndrome (SIRS, also called sepsis), induced by infection, can lead to acute respiratory distress syndrome (ARDS), multiorgan dysfunction syndrome (MODS) or even death[1]. Nuclear factor-κB (NF-κB), as a transcription regulation factor, plays an important part in sepsis and can regulate many cytokines such as tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6)[2]. In this study, the SIRS rat model was established to investigate the role of Shenfu Injection (参附注射液, SFI) by observing its effect on the activity of NF-κB in blood mononuclear cells, the levels of TNF-α and IL-6 in plasma and the pathological changes of the lungs and liver.

METHODS

Animals, Regents and Instruments

Forty-five male Wistar rats, 180 to 220 g (supplied by Animal Center Tongji Medical College, Huazhong University of Science and Technology); lipopolysaccharide (LPS, Ecoli.O127:B8, Sigma, USA); nuclear extracts (Active Motif, USA); TNF-α and IL-6 ELISA Kits (Jingmei Company, China); NF-κB TransAM ELISA Kits (Active Motif, USA); lymphocytes separation medium (Shanghai, China); SFI Injection (Ya'an Sanjiu Pharmaceutics Co., Ltd., China, containing

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0.8 mg of ginsenosides and 0.1 mg of aconitine per milliliter); desktop high speed low temperature centrifuge (TGL.16G, Shanghai Anting Factory of Scientific Instrument, China); desktop low speed centrifuge (TDL-5-A, Shanghai Anting Factory of Scientific Instrument, China); optical microscope (XSZ-D2, Olympus, Japan); and enzyme-linked immunosorbent analyzer (230SEL, Organon Teknika, Holland).

Grouping, Modeling and Treatment

Rats were randomly divided into 3 groups: the sham operative control group (control group, *n*=5), the SIRS model group (model group, *n*=20) and the SFI treatment group (SFI group, *n*=20). The establishment of the SIRS model is referred to the literature[3], which is as follows. After an intraperitoneal injection of 2% pentobarbital sodium (45 mg/kg), the right external jugular vein and the right femoral artery were separated and cannulated, and the latter was used for monitoring the blood pressure and blood sample collection.

LPS (12 mg/kg, 6 mg/mL) was injected to rats except for those in the control group through the external jugular vein, and SFI (10 mL/kg) was given to those in the SFI group only once through intraperitoneal injection, while the normal saline (10 mL/kg) was given to those in the model group. For those in the control group, normal saline was given through the external jugular vein (2 mL/kg) and through intraperitoneal injection (10 mL/kg). Then, rats in the model group and SFI group were divided into 4 subgroups according the time points, i.e., 1 h, 2 h, 4 h and 6 h subgroups, 5 in each group.

The blood sample (5 mL) was withdrawn at each time point, and stored in EDTA anticoagulant tube. The 2 mL of the sample was used for separating plasma, 2 mL for separating mononuclear cells, and the rest for leukocyte count.

Separation of Mononuclear Cells

Blood mononuclear cells were obtained using Ficoll density gradient centrifugation with concrete manipulation according to the instructions in the kits.

Extract of Nucleoprotein

The nucleoprotein was extracted according to the literature[4] using a nuclear extract kit. A total of 5 μg nucleoprotein was extracted from each sample for the NF-κB binding assay.

Activity and Specificity Analysis of NF-κB

The activity of NF-κB in mononuclear cells was measured with TransAM ELISA Kit according to the literature[4].

Determination of TNF-α and IL-6 Levels in Plasma

The levels of TNF-α and IL-6 were determined using ELISA according to the instructions in the kits.

Histological Analysis of Lungs and Liver

The rats in the control group, the 6-h model subgroup and the 6-h SFI subgroup were sacrificed. The samples were fixed in formalin, embedded in paraffin, and made into paraffin sections. After staining with hematoxylin and eosin (HE), the sections were observed under an optical microscope.

Statistical Analysis

Data were expressed as the mean ± standard deviation. The data were analyzed with One-way ANOVA and *q*-test using SPSS 12.0 software. *P* value less than 0.05 was taken as statistically significant.

RESULTS

SIRS Model Evaluation

The rats in the model group manifested the following: the rectal temperature was increased or decreased by 1 ℃; the heart rate was increased by 50% compared with that in the control group; the respiration rate was increased to over twice the level in the control group; the leukocyte count was twice more than or 50% less than that in the control group, indicating that the SIRS model was successfully established[5].

Comparison of NF-κB Activity in Mononuclear Cells, Plasma Levels of TNF-α and IL-6 Among Groups

The NF-κB activities in mononuclear cells and plasma levels of TNF-α in the model group were all higher than those in the control group at corresponding time points (all *P*<0.01), and the peak values occurred at 2 h after modeling. Compared with the model group, both indexes were lower in the SFI group at corresponding time points (all *P*<0.01, Table 1).

The plasma IL-6 levels in the model group increased gradually as time went by, showing significant difference when compared with those of the control group at corresponding time points (all *P*<0.01). The

Table 1. Comparison of NF-κB Activity in Mononuclear Cells, Plasma Levels of TNF-α and IL-6 among Groups ($\bar{x} \pm s$)

Group	*n*	Time	NF-κB (Absorbency)	TNF-α (ng/L)	IL-6 (ng/L)
Control	5	–	0.112 ± 0.017	55.857 ± 19.159	328.000 ± 21.237
Model	5	1 h	0.243 ± 0.022*	465.688 ± 26.600*	1947.200 ± 21.845*
	5	2 h	0.604 ± 0.020*	1644.770 ± 25.920*	3553.400 ± 21.893*
	5	4 h	0.442 ± 0.019*	848.474 ± 24.263*	4187.800 ± 24.448*
	5	6 h	0.296 ± 0.018*	489.217 ± 23.923*	6358.800 ± 23.858*
SFI	5	1 h	0.122 ± 0.014△	103.474 ± 18.614△	1083.600 ± 23.965△
	5	2 h	0.297 ± 0.017△	652.769 ± 26.350△	2008.200 ± 19.967△
	5	4 h	0.187 ± 0.020△	406.084 ± 23.076△	2607.200 ± 16.873△
	5	6 h	0.125 ± 0.019△	123.530 ± 27.454△	3249.000 ± 19.736△

Notes: *P<0.01, compared with the control group; △P<0.01, compared with the model group at the same time points

IL-6 levels in the SFI group decreased significantly as compared with those in the Model group at all time points (all P<0.01, Table 1).

Histopathologic Changes of Lungs and Liver

In the control group, there were no pulmonary alveoli hemorrhage and edema. The alveolar septum was normal with no thickening, and inflammatory cell infiltration was not found in the lung tissue. There were no ectasis and congestion in the liver sinus hepaticus. No local necrosis of liver cells was observed (Figure 1A, 1D).

In the 6-h model group, hemorrhage and edema in the pulmonary alveoli, diffused thickening, edema and inflammatory cell infiltration in the alveolar septum were found in the lung tissue. Positive findings in the liver included sinus ectasis, congestion and local necrosis of liver cells (Figure 1B, 1E).

Compared with the 6-h model group, the injuries of the lungs and liver were alleviated significantly in the 6-h SFI group (Figure 1C, 1F).

DISCUSSION

Infection could induce the early protective inflammatory response, but an excessive or uncontrollable

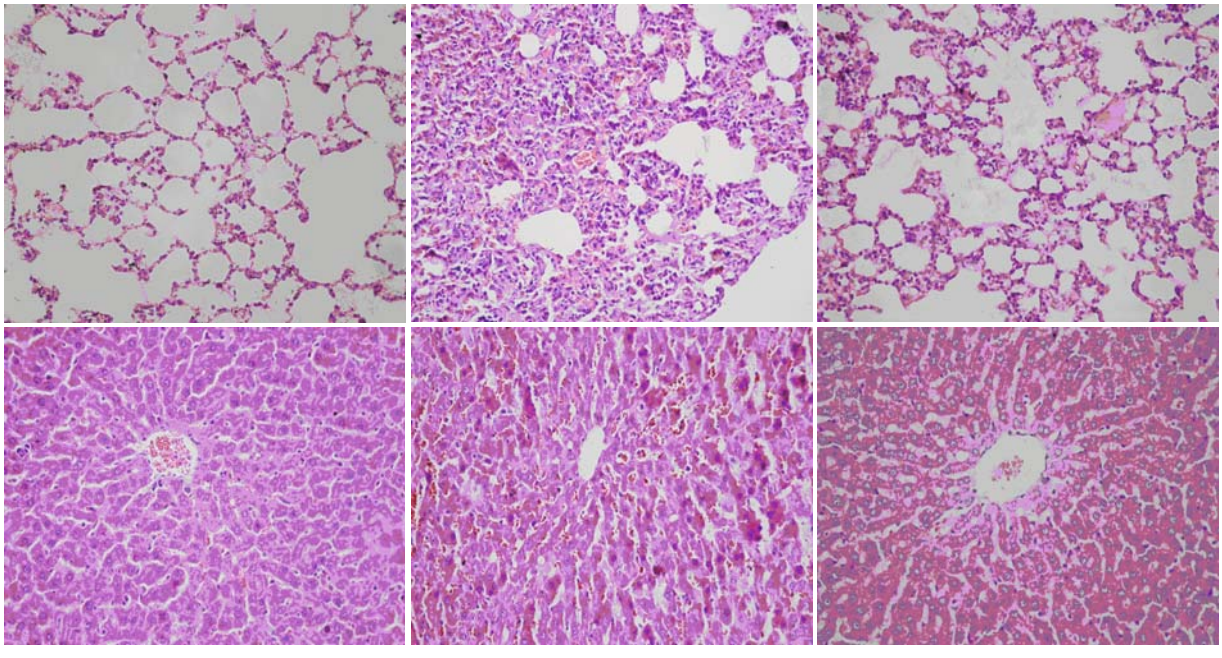

Figure 1. Histopathological Observation of Lungs and Liver

Notes: Lungs (HE, ×200): A: the control group; B: the 6-h model group; C: the 6-h SF group; Liver (HE, ×400): D: the control group; E: the 6-h model group; F: the 6-h SF group

inflammatory response could result in "systemic waterfall-like inflammatory cascade response", thus causing SIRS and MODS. The bacterial endotoxin could activate the signal transduction system, induce the translocation of NF-κB and activate the proinflammatory factor code genes, leading to the synthesis and release of harmful cytokines, such as TNF-α, IL-6, etc. These cytokines could cause the leukomonocyte infiltration in the lung tissues, and the inflammatory factors induced by leukomonocytes could cause hypotension, acidosis and tissue damage, eventually leading to organ dysfunction and death[6].

NF-κB, as one of the key transcription factors, regulates gene expression of inflammatory factors. The binding sites of NF-κB exist on the gene promoter of many cytokines including induced niric oxide synthase, TNF-α and IL-6. Many studies showed that the increased NF-κB activity was relevant to poor prognosis in patients with sepsis or SIRS[7]. Paterson, et al[8] found that the NF-κB activity of peripheral blood monocytes and neutrophile granulocytes was markedly increased in patients with SIRS, especially in those who died of SIRS (in whom the activity was much higher than that in the surviving group).

Activation of NF-κB could initiate and regulate the gene expression of a series of inflammatory factors involved in inflammatory reactions. Blackwell, et al[9] found that the activity of NF-κB in the lung tissue was increased in a short time in a rat hemorrhagic shock model, which was followed by obvious increases in various cytokines. TNF-α, as an early produced cytokine and an important proinflammatory factor medicated by NF-κB, is a principal mediator activating the cytokine cascade responses in inflammatory reaction. TNF-α appears quite early in the cycle and reaches its peak rapidly, and thus initiating a series of cytokines, such as the synthesis and release of IL-6. The excessive release of these pro-inflammatory mediators triggers an irreversible inflammatory reaction in sepsis[7]. Studies showed that the intravenous administration of TNF-α could simulate sepsis and the response of severe injury in animal models[10]. Blockade of IL-1β or TNF-α could attenuate the inflammatory reaction. In addition, TNF-α could also activate NF-κB on the other hand, producing excessive-releasing of inflammatory mediators like waterfall cascade reaction, and resulting in sepsis or even MODS.

IL-6 is a tardive cytokine. The elevation of plasma IL-6 was delayed relative to that of TNF-α in infection shock[11]. It was demonstrated that serum IL-6 may be taken as a mark of the activation of cytokine cascade reaction, reflecting the relationship between the host inflammatory response and the degree of the disease, as well as an indicator for the prognosis judgment in sepsis[10].

SFI consists of ginsenoside and aconitine. Modern pharmacological studies show that SFI has direct actions of inactivating xanthine oxidase, fighting against oxygen free radicals, improving the hemorrheology and immune function as well as the central nervous system function, protecting vascular endothelial cells, anti-inflammation and enhancing the hypoxia tolerance[12]. However, there have been no reports on SFI's effects on the activity of NF-κB in mononuclear cells of SIRS rats. This study showed that the activation of NF-κB in SIRS rats could lead to the increased plasma levels of TNF-α and IL-6 and aggravate the lung and liver injury. After SFI administration, the activity of NF-κB was obviously inhibited, accompanied with decreased plasma levels of TNF-α and IL-6 and significantly alleviated lung and liver injury. Therefore, the protective role of SFI in SIRS rats might be relevant to its inhibitory effect on the activity of NF-κB.

In summary, SFI can decrease the expression of TNF-α and IL-6 and alleviate lung and liver injury by inhibiting NF-κB activation, thus playing a protective role in SIRS rats.

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