Letter to the Editor

Protective Effects of Ginsenoside Rb1 on Septic Rats and Its Mechanism*

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This study aims to observe the protective effects of ginsenoside Rb1 on liver and lung in rats with septic shock and reveal its mechanism. Rats were randomly divided into three groups: sham, cecal ligation and puncture (CLP), and CLP with ginsenoside Rb1. Then, the survival rate, arterial blood pressure, TLR4 mRNA, and TNF-α levels were determined. The liver and lung tissues were stained with hematoxylin-eosin (HE). The overall survival rate of the Rb1 group was significantly higher than that of the CLP group. Mean arterial blood pressure went down in both the CLP and Rb1 groups after CLP, and there was a significant difference both in the sham and Rb1 groups when compared with the CLP group. The Rb1 treatment group had markedly lower TLR4 mRNA expression and TNF-α levels than the CLP group. In the CLP group, pathology showed swelling, degeneration, necrosis, and neutrophil infiltration in the liver and alveolar epithelial cells. However, in the Rb1 group, there was mild degeneration and slight neutrophil infiltration, but no obvious necrosis. Rb1 may improve the survival rate, ameliorate arterial blood pressure, and protect the liver and lung in septic shock rats by downregulating the expression of TLR4 mRNA and inhibiting the production of TNF-α.

Sepsis is a systemic, deleterious host response to invasive infection leading to acute organ dysfunction. Multiple organ dysfunction syndrome (MODS) is the identified failure of critical organ function and is the hallmark of severe sepsis and septic shock. The intensity of MODS is directly correlated to mortality and MODS is the main cause of death in patients with severe sepsis[1]. Severe sepsis and septic shock are the major causes of morbidity and mortality in critically ill patients, and affect millions of people worldwide each year.

Ginsenosides are the most important ingredient of *Panax ginseng* and are known to possess many pharmacological and biological properties including anti-aging, anti-carcinogenic, anti-oxidation, anti-inflammation, and anti-fatigue effects. Ginsenoside Rb1, one of the principle active ingredients of ginseng, exerts multiple pharmacological activities. It has showed neuroprotective effects against ischemia, glutamate neurotoxicity, seizures, motor impairment, and cell loss in the striatum[2]. However, no data have yet been reported concerning the effect of Rb1 on vital organs in rats with septic shock. In this study we investigated whether Rb1 could protect the vital organs in rats with septic shock and explored its mechanism.

Eighty-four Sprague Dawley (SD) rats were randomly divided into three groups: sham, cecal ligation and puncture (CLP) alone, and CLP treated with 0.04 mg/g ginsenoside Rb1 (n=28 rats each). Sixteen rats from each group were randomly chosen for observation of survival for 7 d. Six rats were randomly chosen for recording of arterial blood pressure. The remaining six rats were killed after CLP and blood was collected for arterial blood gas analysis, observation of the main pathological changes, determination of the expression of TLR4 mRNA in myocardial tissue, and the concentration of plasma TNF-α. In the ginsenoside Rb1 group, rats were intraperitoneally injected with 0.04 mg/g ginsenoside Rb1 30 min before performing CLP and then received an additional injection every 8 h, a total of three times. Sham and CLP-alone rats received the same volume of saline.

Data processing was performed using SPSS version 13.0. ANOVA and Student-Newman-Keuls q test were used for statistical analysis to compare measurement data among all groups. The survival comparisons between groups were performed using the Kaplan-Meier method. The results were recorded as mean±SD ($x\pm s$). Differences were considered significant with probability values of less than 0.05.

We have previously used CLP to investigate

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clinical sepsis and septic shock, which is marked by hypoglycemia, hypoinsulinemia, and hypodynamic circulation with markedly increased lactate levels[3]. Therefore, we recorded the survival rate and time over 7 d, measured the mean arterial blood pressure, and carried out arterial blood gas analysis in CLP rats with or without ginsenoside Rb1 treatment. The 24, 36, and 48 h survival rates of the sham group were 100% (16/16), 100% (16/16), and 100% (16/16), respectively, those of CLP group were 50.0% (8/16), 43.8% (7/16), and 37.5% (6/16), respectively and those of ginsenoside Rb1 group were 81.3% (13/16), 63.3% (10/16), and 56.25% (9/16), respectively. At each time point, the survival rate in the ginsenoside Rb1 group was higher than at the corresponding time point in the CLP group. The mean survival time was 7 d in the sham group, 1 d in the CLP group, and 6 d in the ginsenoside Rb1 group. Compared with the CLP group, the survival rate of the Rb1 group was significantly higher (P<0.01). Mean arterial blood pressure decreased continuously in both the CLP and Rb1 groups after CLP operation, and reached its lowest value (38.50±8.67 and 65.33±6.09 mmHg, respectively) at 10 h. At 1, 2, and 3 h, when compared with CLP group, there was no significant difference both in the sham and Rb1 groups (P>0.05). However, at 4, 5, 6, 7, 8, 9, and 10 h, there were significant differences in both the sham and Rb1 groups compared with the CLP group (P<0.05 and P<0.01, respectively) (Table 1). Significantly higher arterial blood PO_2, higher PCO_2, and lower pH levels were observed in the CLP group when compared with the sham group. The arterial blood PO_2 and pH in the Rb1 group were markedly higher than those in the CLP group (P<0.05) (Table 2). Our studies have demonstrated that ginsenoside Rb1 could reverse the hypotension caused by infection and ameliorate arterial PO_2 and pH, thus dramatically improving the survival rate of septic shock in rats and prolonging their survival time.

If untreated, rats subjected to CLP may become ill and die from multiple organ failure. The lung is particularly sensitive to endotoxin and is usually the first organ to fail, followed by the liver[4]. Accordingly, we observed the main pathological changes of lung and liver tissues with or without ginsenoside Rb1 treatment. Sham rats showed intact hepatocytes, normal liver lobules, and hepatic sinusoid without hypertrophy or inflammatory cell infiltration (Figure 1A). Livers from CLP rats had swollen hepatocytes, vesicular fatty degeneration, punctiform cellular necrosis with focal abscesses in portal areas, dilated sinusoids, and neutrophil accumulation in the vessel walls (Figure 1B). CLP rats treated with ginsenoside Rb1 showed cloudily swollen hepatocytes, slight fatty degeneration, few infiltrating neutrophils in hepatic sinusoids and no obvious necrosis (Figure 1C). Moreover, lung pathology of sham rats showed normal alveolus, alveolar epithelial cells, alveolar septum, no capillary congestion, no hemorrhaging or edema in the pulmonary interstitium, and no inflammatory cell infiltration (Figure 2A). CLP rats showed swollen alveolar epithelial cells, thickened alveolar septum, expansion and congestion in the capillaries with microthrombosis, congestion and edema in the pulmonary interstitium, diffuse infiltrating neutrophils, and diminished alveolar space with heavy inflammatory exudation (Figure 2B). In comparison, CLP rats treated with ginsenoside Rb1 showed slight neutrophil infiltration in small vessels and capillaries, wild edema in pulmonary interstitial, little thickened alveolar septum, and no exudants in the

Table 2. Arterial Blood Gas nalysis in Three Groups (n=6, x±s)

Group	pH	PCO_2 (mmHg)	PO_2 (mmHg)
Sham	7.30±0.02	32.0±0.4	107.3±0.3
CLP	7.13±0.05*	43.2±1.3*	95.1±1.9*
Rb1	7.27±0.07#	36.7±0.9	103.6±0.5#

Note. *P<0.05, compared with the sham group; #P<0.05, compared with the CLP group.

Table 1. Changes in MAP in Three Groups Over the Observation Period (mmHg, n=6, x±s)

Group	Time (after CLP)									
	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h	9 h	10 h
Sham	131.33±3.98	125.67±7.76	115.67±7.45	111.50±7.53	110.00±8.05	102.17±8.61	93.17±6.27	83.83±4.17	78.67±6.35	73.67±5.72
CLP	132.00±4.98	124.33±4.59	114.33±4.59	94.33±3.14*	90.17±2.93*	78.83±4.96*	63.50±3.62*	47.67±5.61*	44.67±8.73*	38.50±8.67*
Rb1	133.83±2.79	123.00±3.10	111.83±3.06	107.17±4.71##	100.83±8.11#	92.67±11.31#	86.00±8.85##	77.17±11.09##	69.33±9.99##	65.33±6.09##

Note. *P<0.01, compared with sham group; #P<0.05, ##P<0.01, compared with the CLP group.

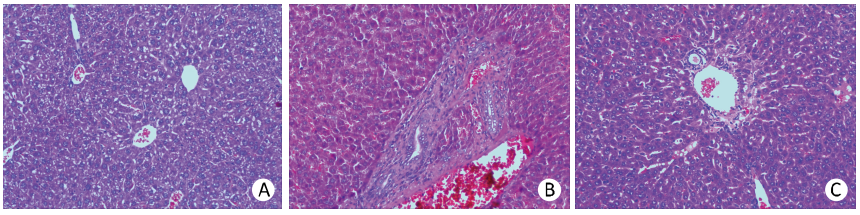

Figure 1. Pathological changes in liver tissues (HE staining, ×200). A, Sham group; B, Cecal ligation and puncture group; C, ginsenoside Rb1 (0.04 mg/g, wt, i.p) group.

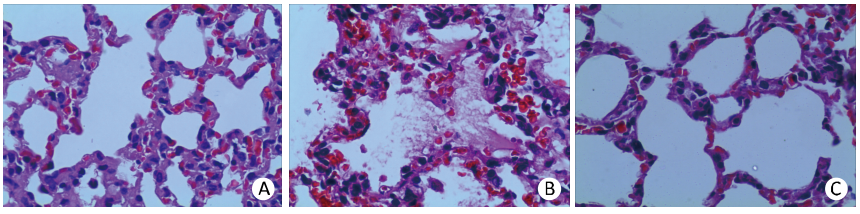

Figure 2. Pathological changes in lung tissues (HE staining, ×200). A, Sham group; B, Cecal ligation and puncture group; C, ginsenoside Rb1 (0.04 mg/g) group.

pulmonary interstitium (Figure 2C). Therefore, ginsenoside Rb1 could relieve liver and lung injury in septic shock rats.

Experiments have been carried out to investigate the potential molecular mechanism of the protective effect of Rb1. TLR4 is the most ancient of the toll-like receptors (TLRs), with an origination date of before vertebrate life. TLR4 is activated by ligands, inducing increased expression of cytokines including IL-6 and TNF-α. We observed that TLR4 mRNA expression in myocardial tissues was higher after CLP in septic rats. TLR4 mRNA expression in myocardial tissues was higher 4 h after CLP (0.737±0.025), and the difference was significant compared with the sham group (0.268±0.006, P<0.01). Therefore, TLR4 expression is upregulated via signal transduction pathways in the rat cardiomyocytes, inducing the production of inflammatory molecules.

Systemic sepsis releases several cytokines, among which TNF-α has emerged as the key cytokine causing septic shock[5]. TNF-α expression was characterized by an immediate increase in affected structures starting as soon as 1 h after stimuli. Peak levels are detected around 24 h, sustain for about 3 d, and then decrease in many cases, occasionally to baseline levels[6]. Accumulating evidence indicates that the plasma concentration of TNF-α is correlated with the onset and severity of multiple organ dysfunction syndrome (MODS)[7]. Our studies also found that the plasma TNF-α was markedly higher 4 h after CLP in septic rats. A concentration of 108.56±14.05 pg/mL was observed in sham rats and CLP caused markedly higher TNF-α concentrations at 4 h (523.21±83.33, P<0.01). TNF-α can incite the production of free radicals, nitric oxide, and eicosanoids from various cells that can also produce several pathophysiologic changes, such as myocardial dysfunction and cardiomyocyte death, which is a common clinical manifestation in sepsis and septic shock[8-9].

Ginsenoside Rb1 treatment caused a notably lower expression of TLR4 mRNA in myocardial tissue and concentration of plasma TNF-α compared to CLP alone. Compared with the CLP/saline group, 0.04 mg/g ginsenoside Rb1 administration could lower TLR4 mRNA expression (0.347±0.071, P<0.01) and TNF-α levels (252.10±12.44, P<0.01). Ginsenoside Rb1 might down-regulate TLR4 expression via signal transduction pathways and attenuate the production of TNF-α. Our results are consistent with previous reports by Zhu[10], who demonstrated that Rb1 could inhibit activation of inflammatory cells, downregulate pro-inflammatory cytokines, and suppress a key transcription factor.

We found that pretreatment with ginsenoside Rb1 seemed to have a protective effect on septic shock rats. The mechanism is probably involved in the inhibition of inflammatory molecule production such as TNF-α via suppression of TLR4 mRNA expression. However, it is not yet clear whether Rb1 has any protective effects when given after CLP.

Therefore, it is necessary to further research this field. Ginsenoside Rb1 could be applied in the clinical therapy for sepsis and septic shock in the future.

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REFERENCES

1. Semeraro N, Ammollo CT, Semeraro F, et al. Sepsis, thrombosis and organ dysfunction. Thromb Res, 2012; 129, 290-5.

2. Lee SH, Hur J, and Lee EH. Ginsenoside rb1 modulates level of monoamine neurotransmitters in mice frontal cortex and cerebellum in response to immobilization stress. Biomol Ther (Seoul), 2012; 20, 482-6.
3. William J Hubbard, Mashkoor Choudhry, et al. CECAL LIGATION AND PUNCTURE. SHOCK, 2005; 24, Suppl. 1, 52-7.
4. Connor AJ, Chen LC, Joseph LB, et al. Distinct responses of lung and liver macrophages to acute endotoxemia: role of toll-like receptor 4. Exp Mol Pathol, 2013; 94, 216-27.
5. Kothari N, Bogra J, Abbas H, et al. Tumor necrosis factor gene polymorphism results in high TNF level in sepsis and septic shock. Cytokine, 2013; 61, 676-81.
6. Pablo Andrade, Veerle Visser-Vandewalle, Carolin Hoffmann, et al. Role of TNF-alpha during central sensitization in preclinical studies. Neurol Sci, 2011; 32, 757-71.
7. Das UN. Critical advances in septicemia and septic shock. Crit Care, 2000; 4, 290-6.
8. Undurti N Das. Critical advances in septicemia and septic shock. Crit Care, 2000; 4, 290-6.
9. Kumar A, Haery C, and Parrillo JE. Myocardial dysfunction in septic shock: part I. Clinical manifestation of cardiovascular dysfunction. J Cardiothorac Vasc Anesth, 2001; 15, 364-76.
10. Zhu J, Jiang Y, Wu L, et al. Suppression of local inflammation contributes to the neuroprotective effect ofginsenoside Rb1 in rats with cerebral ischemia. Neuroscience, 2012; 202, 342-51.