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The expression changes and correlation analysis of high mobility group box-1 and tissue factor in the serum of rats with sepsis

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Abstract. – OBJECTIVE: To investigate the expression changes of high mobility group box-1 (HMGB-1) and tissue factor (TF) and their correlation in the serum of sepsis rat models.

MATERIALS AND METHODS: 30 rats were divided into the sham-operated group, 15 rats were in the control group. The cecal ligation and puncture method was used to make the animal model with abdominal infection induced by sepsis. There were 15 rats in the sepsis group among which they were divided into 3 subgroups at different time points after modeling (after 6 hours, 12 hours, 24 hours). Cardiac function indicators of the rats in each subgroup were monitored, including heart rate (HR), left ventricular end-diastolic pressure (LVEDP) and left ventricular developed pressure (LVDP), and enzyme-linked immunosorbent assay (ELISA) was used to test the changes of the expression levels of HMGB-1 and TF in the serum of the rats after 6 hours, 12 hours, 24 hours. Pearson correlation analysis was used to analyze the correlation between HMGB-1 and TF.

RESULTS: HR and LVEDP of the rats in the sepsis group were significantly higher than those of the rats in the control group. The differences were statistically significant ($p<0.050$). LVDP of the rats in the sepsis group was markedly lower than that of the rats in the control group. The differences were statistically significant ($p<0.050$). The expressions of HMGB-1 and TF of the rats in the subgroups of the sepsis group were higher than those of the rats in the control group after 6 hours, 12 hours, 24 hours; the expression levels of HMGB-1 and TF of the rats with sepsis increased with time. The differences were statistically significant ($p<0.050$). When the expressions of HMGB-1 and TF of the rats in the sepsis group were compared with each other within the group the differences were significantly different ($p<0.050$). The expressions of HMGB-1 and TF in the subgroups at the 24th hour were significantly higher than those at the 6th hour. The differences were statistically significant ($p<0.050$). The differences in the expression of TF of the rats in the control group were not statistically significant ($p>0.050$). There was a significant positive correlation between HMGB-1 and TF of the rats in the sepsis group ($r=0.772$, $p=0.002$).

CONCLUSIONS: The expression levels of HMGB-1 and TF of the rats with sepsis gradually increased with time, and the level of HMGB-1 was positively correlated with the level of TF.

Key Words:
Rat with sepsis, Cardiac function, HMGB-1, TF, Expression change, Correlation analysis.

Introduction

Sepsis is a systemic inflammatory response caused by bacterial, fungal or viral infections, which results in functional disorders, including inflammation function, coagulation function, immunologic function and endothelial function, to develop into septic shock[1,2]. When the immune system overreacts to existing infections, the inflammatory response may lead to dysfunction of vital organs, including lung, heart, kidney and liver, and lead to multiple organ dysfunction syndrome, which causes that patients with serious illness are difficultly cured, and can cause their death[3]. In recent years, much attention has been

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paid to the treatment of sepsis and its complications and progress has been achieved in the medical field, but in intensive care units, the mortality of patients with septic shock and multiple organ failures caused by systemic infection is as high as 30% to 50%[4]. Cardiac function insufficiency is one of the serious complications of sepsis[5]. At present, the mechanism of the injury of cardiac function in sepsis has not been completely clarified, but cardiomyocyte apoptosis may be an important factor resulting in myocardial suppression in sepsis[6]. High mobility group box-1 (HMGB-1) is a highly conserved inflammatory factor that commonly exists in cell nucleuses; it is involved in activities like the maintenance of the nucleosome structure, the regulation of gene transcription and the regulation of the activity of the steroid hormone receptor. It also mediates a variety of cellular responses, including cell migration, the release of proinflammatory cytokines, tissue repair and angiogenesis[7,8]. It has been reported in literature that HMGB-1 is involved in the inflammatory response of myocardial injury in sepsis[9]. The system activates the formation of coagulation and thrombosis in severe sepsis. Tissue factor (TF) is a receptor which exists in the surface of the transmembrane cell of plasma coagulation factor VII, it is homologous with the cytokine receptor family and is the main trigger of coagulation process[10]. At present, there are a few clinical studies about sepsis, HMGB-1 and TF; thus, the cardiac function of rats with sepsis, the expression changes of HMGB-1 and TF and their correlation in serum were compared in this paper to provide references and ideas for the clinical treatment of sepsis.

Methods and Materials

Experimental Animals

30 Wistar rats of clean grade with the age of 6 weeks, body mass (200-250) g, average body mass (231.23±5.37) g were used. The rats were purchased from the Shanghai Slack Laboratory Animals Co., Ltd., production license SCXK (Hu) 2012-0002. They were fed in a single cage in which the temperature was (24.00±2.00)°C and the humidity was (50.00±5.00)%, with a normal 12-hour circadian rhythm with the condition of freely eating foods and drinking water. This study was approved by the Animal Ethics Committee.

Modeling Methods

After all the rats were adaptively fed for 1 week, the cecal ligation and puncture method was used to establish the rat model with abdominal infection induced by sepsis according to the reports of Antoniak[11]. The rats were randomly divided into the sepsis group and the control group; there were 15 rats in each group. The rats in the two groups were not allowed to eat foods and were allowed to freely drink water in 12 hours before the experiment. Before the operation, the rats were anesthetized with an intraperitoneal injection of pentobarbital sodium, the concentration was 40 mg/kg (Shanghai Shangyao Xinya Pharmaceutical Co., Ltd., SFDA approval number: H31021725). After being anesthetized, the rats were fixed in supine position, the abdomen of the rats was disinfected with iodophor and sterile drapes were whisked onto the rats. This process was performed twice. After the rats were disinfected, a longitudinal incision, the length of which was 1.5 cm, was made in the median line of the anterior abdomen of the hypogastrium of the rats, and the layers were separated until into the abdominal cavity; the cecum was fully exposed and the mesentery was dissociated. The incision of the abdominal wall of the rats in the control group was sutured layer by layer. In the sepsis group, the side wall of the cecum of the rats was circlewise ligated with 3# suture line at the distal end of the ileocecal valve. Then, 16# needle was used to pass through the cecum 3 times at 1 cm of ligation place. After this step, the cecum was extruded to make sure that a small amount of feces outflowed; then, the abdominal cavity was sutured layer by layer. After the operation, the rats in the two groups were given a subcutaneous injection of sterile normal saline with a concentration of 30 ml/kg in the abdomen to supplement the body fluid, and the rats were given free access to eating foods and drinking water.

Test Methods

5 rats were randomly and respectively selected from the two groups after 6 hours, 12 hours and 24 hours after modeling. They were abdominally anesthetized with pentobarbital sodium with a concentration of 30 mg/kg, the left common carotid artery of the rats was intubated and connected to the biological signal collection and processing system (Beijing Zhongshi Dichuang Technology Co., Ltd, Beijing, China); the indicators, including heart rate (HR), left

ventricular end-diastolic pressure (LVEDP) and left ventricular developed pressure (LVDP), were recorded and compared. Then, 5 ml of the blood was taken and a centrifuge (Changsha Xiangzhi Centrifuge Instrument Co., Ltd.) was used to centrifuge the blood at a speed of 3000 r/min for 15 min to separate the serum. Enzyme-linked immunosorbent assay (ELISA) was used to test the changes of the expression levels of HMGB-1 and TF in the serum of the rats strictly according to rat HMGB-1 ELISA test kit (Shanghai Chunshi Biotechnology Co., Ltd., Item No.: CS-ELISA 5825, Shanghai, China) and rat TF ELISA test kit (Shanghai Fanke Biotechnology Co., Ltd., Item No.: FK-mu1618, Shanghai, China). After the study, the rats were killed by decapitation in a state of anesthesia.

Statistical Analysis

SPSS 17.1 (SPSS Inc., Chicago, IL, USA) software system was used to carry out the statistical analysis. The expression levels of HR, LVEDP, LVDP, HMGB-1, and TF were expressed in the form of mean value ± standard deviation, *t*-test was used to analyze the differences between the two groups. Pearson correlation analysis was used to analyze the correlation between the level of HMGB-1 and the level of TF. When $p<0.05$, the differences were statistically significant.

Results

Modeling Results

15 rats were used to establish the sepsis rat model, among which 13 rats were successfully modeled, and the success rate of the modeling was 86.67%.

The Comparison of HR, LVEDP and LVDP of the Rats Between the Sepsis Group and the Control Group

HR of the rats in the sepsis group was (352±31) times/min, HR of the rats in the control group was (314±22) times/min, HR of the rats in the sepsis group was significantly higher than that of the rats in the control group. The difference was statistically significant ($t=3.779$, $p=0.001$). LVEDP of the rats in the sepsis group was (7.36±0.61) mmHg, LVEDP of the rats in the control group was (4.27±0.52) mmHg, LVEDP of the rats in the sepsis group was markedly higher than that of the rats in the control group. The difference was statistically significant ($t=14.480$, $p<0.001$). LVDP of the rats in the sepsis group was (78.6±8.4) mmHg, LVDP of the rats in the control group was (92.5±9.1) mmHg, LVDP of the rats in the sepsis group was remarkably lower than that of the rats in the control group, the difference was statistically significant ($t=4.176$, $p<0.001$; Figure 1-3).

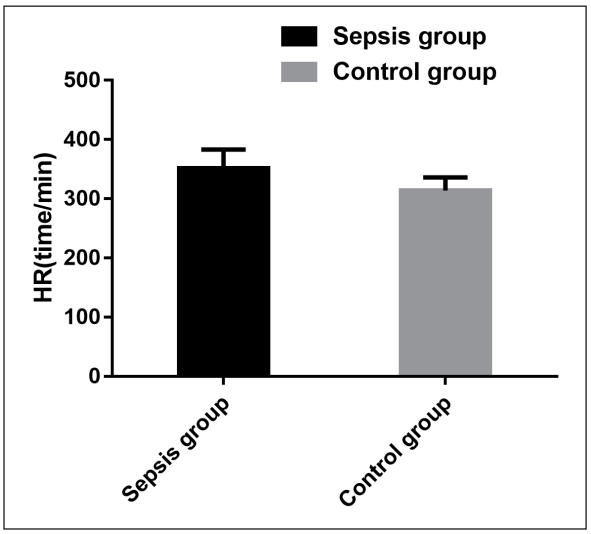

Figure 1. The comparison of HR of the rats between the sepsis group and the control group. HR of the rats in the sepsis group is significantly higher than that of the rats in the control group, the differences are statistically significant ($t=3.779$, $p=0.001$).

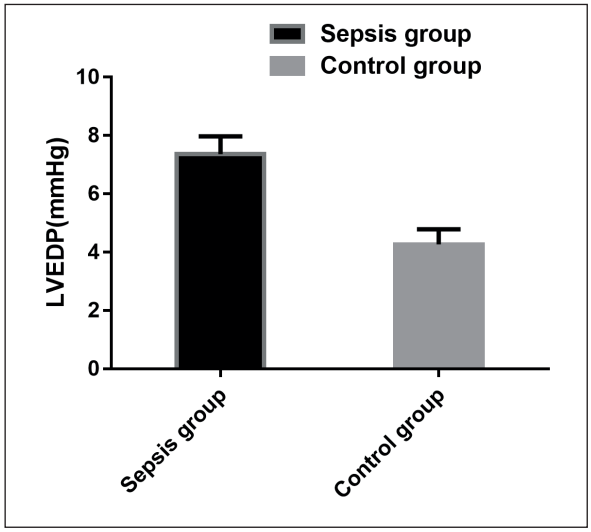

Figure 2. The comparison of LVEDP of the rats between the sepsis group and the control group. LVEDP of the rats in the sepsis group is markedly higher than that of the rats in the control group, the differences are statistically significant ($t=14.480$, $p<0.001$).

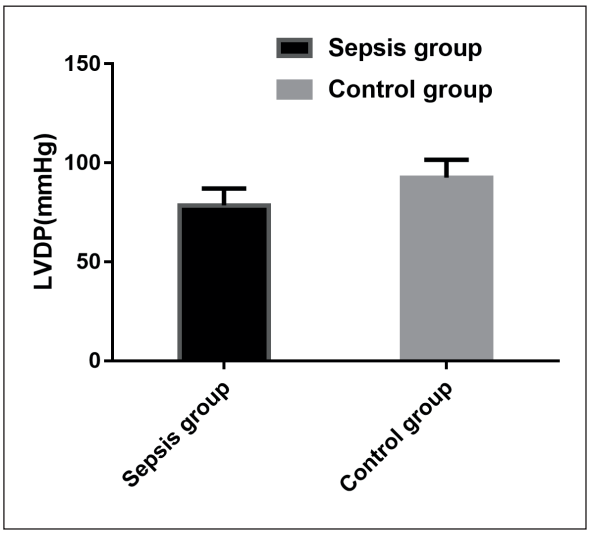

Figure 3. The comparison of LVDP of the rats between the sepsis group and the control group. LVDP of the rats in the sepsis group is remarkably lower than that of the rats in the control group, the differences are statistically significant (t=4.176, p<0.001).

The Comparison of the Changes of the Expression Levels of HMGB-1 of the Rats Between the Sepsis Group and the Control Group

The expression of HMGB-1 of the rats in the subgroups of the sepsis group was higher than that in the control group at the 6th hour. The difference was statistically significant (t=5.425, p=0.002). The expression of HMGB-1 of the rats in the subgroups of the sepsis group was higher than that in the control group at the 12th hour. The difference was statistically significant (t=6.058, p<0.001). The expression of HMGB-1 of the rats in the subgroups of the sepsis group was higher than that in the control group at the 24th hour. The difference was statistically significant (t=7.017, p<0.001). When the expression of HMGB-1 of the rats in the sepsis group was compared with each other in the groups the differences were statistically significant (F=4.303, p=0.045), the expression of HMGB-1 of the rats in the subgroups at the 24th hour was significantly higher than that at the 6th hour and the difference was statistically significant (p<0.050). The differences in the expression of HMGB-1 of the rats in the control group were not statistically significant (p>0.050; Table I).

The Comparison of the Changes of the Expression Levels of TF of the Rats between the Sepsis Group and the Control Group

The differences in the expression levels of TF of the rats between the sepsis group and the control group were not statistically significant (p>0.050). The expression of TF of the rats in the subgroups of the sepsis group was higher than that in the control group at the 12th hour; the difference was statistically significant (t=2.364, p=0.046). The expression of TF of the rats in the subgroups of the sepsis group was higher than that in the control group at the 24th hour; the difference was statistically significant (t=3.719, p=0.006). When the expression of TF of the rats in the sepsis group was compared with each other in the groups, the differences were statistically significant (F=4.416, p=0.042), the expression of TF of the rats in the subgroups at the 24th hour was significantly higher than that at the 6th hour; the difference was statistically significant (p<0.050). The differences in the expression of TF of the rats in the control group were not statistically significant (p>0.050; Table II).

The Correlation Analysis Between HMGB-1 and TF of the Rats in the Sepsis Group

There was a significant positive correlation between HMGB-1 and TF of the rats in the sepsis group (r=0.772, p=0.002; Figure 4).

Table I. The changes of the expression levels of HMGB-1 of the rats in the sepsis group and the control group (ng/L).

	No	The sepsis group	No	The control group	t	p
6h	3	165.29±10.36	5	128.65±8.64	5.425	0.002
12h	5	171.42±11.56	5	132.19±8.72	6.058	<0.001
24h	5	189.37±14.13*	5	136.27±9.31	7.017	<0.001
F		4.303		0.919		
p		0.045		0.425		

Note: *compared with that at the 6th hour, the differences were statistically significant (p<0.050).

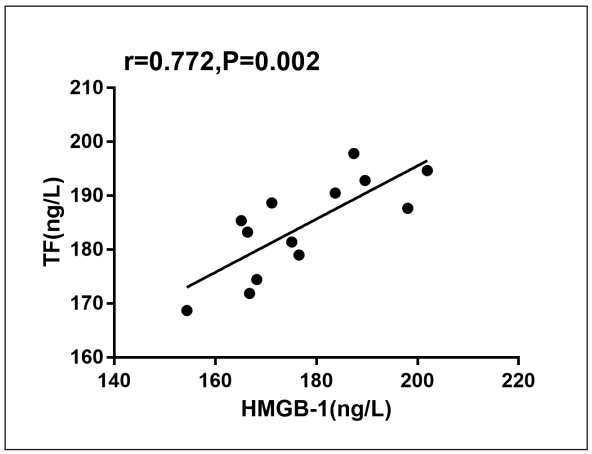

Figure 4. The correlation analysis between HMGB-1 and TF of the rats in the sepsis group. Pearson analysis shows that there is a significant positive correlation between HMGB-1 and TF of the rats in the sepsis group (r=0.772, p=0.002).

Discussion

Sepsis is a physiological, pathological, and biochemical abnormal syndrome caused by infections. Its symptoms include fever, leukocytosis or leukopenia and the decrease of vascular resistance. It often leads to hypotension (septic shock), organ failure (severe sepsis) and death[12]. Although progress has been made in antibiotic therapy, ventilator management, resuscitation strategies and glycemic maintenance, severe sepsis is still the main reason for the death of patients in intensive care units[13]. The morbidity and mortality of sepsis grow at an alarming speed; it endangers the life and health of patients[14,15]. According to the reports of Fleischmann et al[16], sepsis is the main cause that results in death and serious illness worldwide. Patients with sepsis often have long-term physical, psychological and cognitive disorders, which have a serious impact on patients and their family members[17]. This work investigated the effect of sepsis on HMGB-1, TF in the serum of rats and on their cardiac function. In this study, a sepsis rat model was established and analyzed. First, the cardiac function of the rats in the sepsis group and the control group was compared, and it was found that HR and LVEDP of the rats in the sepsis group were significantly higher than those of the rats in the control group, LVDP of the rats in the sepsis group was markedly lower than that of the rats in the control group; the differences were statistically significant. According to the reports of Sordi et al[18], in those patients with sepsis, necrosis and apoptosis of cardiomyocytes result from a large number of cytokines that affect cardiac function in the systemic circulation, thus causing cardiac dysfunction. Especially for those with septic shock, patients have systolic and diastolic dysfunction, as well as the decrease of cardiac output, resulting in the increase of mortality[19,20]. According to Chopra et al[21], which are about rats with sepsis, it is found that LVEDP of rats with sepsis markedly increases compared with that of rats in the sham-operated group. According to Gupta et al[22], it is found that LVEDP and HR remarkably increase in rats with sepsis compared with those of rats in the sham-operated group, which supports the results about cardiac function in our study. However, it has been reported in literature that LVDP in rats with sepsis is lower than that in rats in the sham-operated group[23]. The expressions of HMGB-1 and TF of rats in the sepsis group are markedly higher than those of rats in the control group, and there is a significant positive correlation between HMGB-1 and TF of rats in the sepsis group. Inflammation (anti-inflammatory) dysfunction is an important mechanism of septic shock. The excessive production of inflammatory factors and the bidirectional abnormal state of inflammatory overreaction and inhibition in the body lead to systemic inflammatory response syndrome, which is the

Table II. The changes of the expression levels of TF of the rats in the sepsis group and the control group (ng/L).

	No	The sepsis group	No	The control group	t	*p*
6h	3	153.52±20.26	5	130.94±19.37	1.572	0.167
12h	5	173.74±20.86	5	142.56±20.85	2.364	0.046
24h	5	198.38±21.93*	5	147.17±21.61	3.719	0.006
F		4.416		0.822		
p		0.042		0.463		

Note: *compared with that at the 6th hour, the differences were statistically significant (p<0.050).

main reason of death of patients[24,25]. HMGB-1 is a key inflammatory mediator in sepsis; it can facilitate the release of inflammatory factors and the inflammatory proteins of macrophages, etc. It functions in facilitating an inflammatory response and can aggravate myocardial injury[26]. According to Park et al[27], when the release of HMGB-I is inhibited by β, the survival rate of mice with sepsis can be improved. In the process of multiple organ failures caused by severe sepsis, patients often have abnormal blood coagulation function, and microthrombus mediated by TF pathway and activation of the endothelium are vital[28]. According to the study of Xue et al[29], which are about patients with acute lung injury caused by tissue factors in sepsis, the expression level of TF in the serum of patients with sepsis is significantly higher than that of patients in the health group and TF can be used as an effective biological marker in the diagnosis of sepsis. It is also reported that the injury of TF pathway prevents the abnormality of blood coagulation function in various ways in animal models with sepsis or endotoxemia; the increase of the level of TF in the plasma of patients with sepsis is associated with the increase of the concentration of activation markers of blood coagulation. In the above-mentioned literature, it is speculated that the relationship between inflammation and blood coagulation plays a key role in the pathogenesis of sepsis, microcirculatory failure and multiple organ dysfunction. However, there are a few references about the correlation between HMGB-1 and TF, so our work is innovative and has research values, and more in-depth studies can be carried out later. There are still some shortcomings in this study, the mechanism of sepsis needs to be further studied and there are some differences between animal models and the human body. We will carry out clinical experiments as soon as possible and constantly improve and perfect our investigations to obtain the best results.

Conclusions

We found that the expression levels of HMGB-1 and TF of rats with sepsis increase with time, and there is a significant positive correlation between the levels of HMGB-1 and TF.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

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