Effects of rhubarb on the expression of glucocorticoids receptor and regulation of cellular immunity in burn-induced septic rats

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Abstract

Background: It is important to modulate the expression of glucocorticoids receptor (GR) in tress and maintain the immunity homeostasis in sepsis process. Rhubarb have been shown to have potential effects on anti-inflammatory and immune modulation. The present study was designed to investigate the effects of rhubarb on the expression of GR and cellular immunity in burn-induced septic rats.

Methods: Sixty-six healthy male Sprague Dawley (SD) rats were randomized into sepsis group ($n = 24$), rhubarb group ($n = 24$), and control group ($n = 18$); each group were further randomized into 12, 24, and 72 h subgroups according to different time points. During onset of the sepsis model, the rats in the rhubarb group were infused with 50 mg/kg rhubarb powder dissolved into 1 mL saline through gastric tube, while sepsis and control groups were treated with saline. The binding activity of GR in liver cytosol and binding capacity of GR in peripheral blood leucocyte were analyzed by radiation ligands binding assay. The percentages of $CD4^+$, $CD8^+$,$CD4^+CD25^+$T cells, $CD19^+$B cells as well as natural killer (NK) cells in the lymphocytes in peripheral blood were detected by flow cytometer. For assessing the differences among groups, one-way analysis of variance (ANOVA) with Scheffe multi-comparison techniques were employed. Comparisons between time-based measurements within each group were performed with ANOVA repeated measurement.

Results: The binding activity of GR in liver cytosol and binding capacity of GR in peripheral blood leucocyte were significantly decreased in a time-dependent manner in sepsis group ($t = 23.045$, $P < 0.01$; $t = 24.395$, $P < 0.05$, respectively), which were increased in a time-dependent manner after rhubarb administration ($t = 19.965$, $P < 0.05$; $t = 17.140$, $P < 0.05$, respectively). Twelve hours after sepsis, the percentages of $CD4^+$ T cells, $CD4^+/CD25^+$ T cell ratio, and $CD19^+$ B cells in the peripheral blood were significantly increased in the sepsis group ($t = -3.395$, $P < 0.01$; $t = 2.568$, $P < 0.05$; $t = 2.993$, $P < 0.05$, *vs.* control mice, respectively). However, the percentage of NK cells in the peripheral blood were significantly decreased in the sepsis group ($t = -2.022$, $P < 0.05$, *vs.* control mice). Twelve hours after sepsis, the percentage of $CD8^+$ T cells were significantly decreased in the peripheral blood in the sepsis group ($t = -2.191$, $P < 0.05$, *vs.* control mice) and were significantly increased in the rhubarb group ($t = 2.953$, $P < 0.05$, *vs.* sepsis mice). Seventy-two hours after sepsis, the ratio of $CD4^+/CD25^+$ T cell in peripheral blood were significantly increased in the sepsis group ($t = 2.508$, $P < 0.05$, *vs.* control mice) while were significantly decreased in the rhubarb group ($t = 3.378$, $P < 0.05$, *vs.* control mice). Furthermore, the percentages of $CD19^+$ B cell in peripheral blood were significantly decreased at 72 h in the rhubarb group ($t = 2.041$, $P < 0.05$ *vs.* sepsis group).

Conclusions: Rhubarb might play potential anti-inflammatory and immunomodulatory roles in the sepsis processes.

Keywords: Rhubarb; Sepsis; Cellular immunity; Glucocorticoids receptor

Introduction

Glucocorticoids (GC) is one of the essential anti-inflammatory corticoids in human body. GC plays a crucial role in protecting body from insults under various stress, such as infection, trauma, and hemorrhage. GC showed biological effects mainly through the specific glucocorticoids receptor (GR).[1] GR is a transcriptional regulator which represents as a modular structure and consists of A/B-N-terminal regulatory domain; C-DNA-binding domain; D-hinge region; E-ligand-binding domain, and F-C-terminal domain. The ligand binding domain is the region where glucocorticoids binds. Before binding to GC, the majority of GR is localized in the cytosol and associates with the heat shock protein 90 (hsp90)-containing chaperone complex. The chaperone proteins maintain the receptor that binds glucocorticoids

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with high affinity. Upon binding to GC, GR is dissociated from hsp90-containing chaperone proteins, exposes nuclear localization sequences, and associates with genomic glucocorticoid response elements (GREs) to regulate the transcriptional rate of nearby genes. GR interacts with various proteins, such as transcription factors, cofactor, and modifying enzymes, so its anti-inflammatory capacity is an important action.[2-6]

$CD4^+$,$CD8^+$,$CD4^+CD25^+$T cells, $CD19^+$B cells as well as NK cells play an important role in the elimination of infecting pathogens. For example, $CD4^+$ and $CD8^+$ are the major T cell subsets. $CD4^+$ T cells play a critical role in innate and adaptive immune systems. $CD8^+$ T cells are important for targeted killing of virus infected cells. NK cells are responsible for initiating an inflammatory event and inducing widespread lymphocyte apoptosis. Therefore, T cells and NK cells play a critical role in protecting host against life threatening infections. Patients with Impaired host immune cells are unable to eradicate primary infections and also susceptible to secondary infection during sepsis.[7,8]

Recently, numerous studies have proved the role of immunity dysfunction in septic patients. Therefore, it is important to modulate the expression of GR in stress and maintain the immunity homeostasis in sepsis process. Some traditional Chinese medicine, such as rhubarb, have been shown to have potential effects on anti-inflammatory and immune modulation.[9] The present study was to investigate the effects of rhubarb on the binding activity of GR in liver cytosol, binding capacity of GR in peripheral blood leucocyte, and the percentages of $CD4^+$,$CD8^+$, $CD4^+CD25^+$T cells, $CD19^+$B cells as well as natural killer (NK) cells in the lymphocytes in peripheral blood using the burning induced septic rats, which may indicate the anti-inflammation and immunity modulation effect of rhubarb in burn-induced sepsis.

Methods

Animal model

This study was approved by the Institutional Animal Care and Use Committee of the Second Military Medical University. All animals were provided by Shanghai Slake Experimental Animal Limited Liability Company. Healthy male SD rats weighing 154 to 198 g were fasted for 12 h but water. The rats were anesthetized by intraperitoneal injection with katamine (80 mg/kg). The hair on the back were removed by 10% sodium sulfide. The burning rat model was established by 30% body surface area III degree burns on the back by immerse into boiled water for 12 s and then received intraperitoneal injection of saline 40 mL/kg for fluid resuscitation. The sepsis rat model was established by intraperitoneal injection of endotoxin O111:B4 (5 mg/kg dissolved into 1 mL saline, provided by the Department of Microbiology in Naval Military Medical University) after burning injury. The rats in the control group were anesthetized, hair removed, and immersed into 37°C on the back. We selected all the surviving experimental animals.

Experimental procedures

A total of 66 male rats were randomized into sepsis group ($n = 24$), rhubarb group ($n = 24$), and control group ($n = 18$), each group was divided into three subgroups according to different time point (12, 24, and 72 h). The purified rhubarb (1 g was the equivalent of 4 g raw rhubarb) was produced by rhubarb Institute of Shanghai Xiangshan Traditional Medicine Hospital. The 50 mg/kg purified rhubarb dissolved into 1 mL saline was administrated immediately by gastric tube after burning injury in the rhubarb group; normal saline as a substitute of rhubarb was administrated in the sepsis and the control group. All rats were executed between 7 a.m. and 9 a.m. to avoid the impact of circadian rhythm on GR expression.

Detection of the GR binding activity of rat liver cytosol

The binding activity of GR in liver cytosol was detected by the improved radiation ligands binding assay. Three grams of liver tissues were cut into pieces and rinsed with cold saline, the homogenates were prepared in six volumes (w/v) of Tris buffer, pH 7.40, containing 50 mmol/L potassium phosphate, 10 nmol/L sodium molybdate, 10% glycerol (v/v), 1.50 mmol/L EDTA, and 10 mmol/L ethanol. After the centrifugation (30 min, 35,000 × *g*, 4°C), the super natants 0.3 mL were separated and incubated with 50 μL 1:100 dilution of [3H] Dex (Amersham, UK, specific activity 1.48 TBq/mmol, radiochemical purity >96%) as a total binding and a nonspecific binding. The 2000-fold unlabeled dexamethasone was added and agitated with nonspecific binding mixture and placed for 3 h at 0 to 4°C. The 0.3 mL 5% coated activated carbon (500 mg dextran T70 dissolved into 0.01 mol/L 100 mL Tris pH 7.40, than added with 5 g activated carbon) was added into both binding mixtures for adsorption unbounded [3H] Dex. After centrifugation (5 min, 3000 × *g*), the super natants 0.4 mL were transferred into liquid scintillation cup and added with 0.5 mL scintillation solution (Wallac Product, Allerod, Finland), then placed for 12 h. The specific binding activity was determined by liquid scintillation counter (Microbeta 1450 liquid scintillation counter, Wallac Product). Data were presented as the specific binding activity (total binding minus nonspecific binding) in combination per minute (cpm) and was converted by formula into [3H] Dex specific binding activity (fmol/mg protein). Protein content in the liver cytosols was determined by Lowry method with bovine serum albumin as the standard.

Detection of the GR binding capacity of rat peripheral blood leukocytes

The binding capacity of GR in peripheral blood leucocyte was detected by improved method reported before. The peripheral blood leucocytes were isolated and the concentrations were adjusted to $1–6 \times 10^6$/mL. Then [3H] Dex was added into both total binding and nonspecific binding centrifuge tube to adjusted final concentration as 13 nmol/L. Additional 2000-folds of Dex was added into the nonspecific tube and incubated for 4 h at 24°C. The cold PBS was added to determinate the reaction. The leucocytes were collected on acetate fiber membrane by multiple cell collector and transferred into liquid scintillation cup after

dried, 0.50 mL scintillation solution was added for liquid scintillation counting. Data were presented as the specific binding activity (total binding minus nonspecific binding) in cpm and was converted by formula into [3H] Dex specific binding sites/cells.

Detection of CD4$^+$,CD8$^+$,CD4$^+$CD25$^+$T cells, CD19$^+$B cells and natural killer cell numbers in rat peripheral blood

The number CD4$^+$,CD8$^+$,CD4$^+$CD25$^+$T cells, CD19$^+$B cells, and NK cells in peripheral blood was counted. The 10 μL mice anti-rat CD3,CD4,CD8,CD25,CD19,NK monoclonal antibodies marked by Fluorescein and 10 μL mice anti-rat CD25$^+$ monoclonal antibody marked by PE were added into 100 μL of heparin-anticoagulated peripheral blood, then incubated and protected from light at room temperature for 15 to 20 min. Red blood cell lysis buffer was added and oscillated. Cells were washed once and resuspended, then cells were hemolysis fixed on Couler immune preparation instrument and analyzed with flow cytometer. Five thousand cells were analyzed and the percentages of the CD4$^+$,CD8$^+$,CD4$^+$CD25$^+$T cells, CD19$^+$B cells, and NK cells in the lymphocytes were recorded as well as CD4$^+$/CD25$^+$ and CD8$^+$/CD25$^+$ ratio.

Statistics analysis

Statistical analysis was performed using the SPSS 11.0 (Spss Corp, USA) software program. For assessing the differences among groups, one-way analysis of variance (ANOVA) with Scheffe multi-comparison techniques were employed. Comparisons between time-based measurements within each group were performed with ANOVA repeated measurement. All data were reported as mean ± standard deviation (SD). A P value <0.05 was regarded as significant.

Results

Effects of rhubarb on the binding activity of GR in liver cytosol and the GR binding capacity in peripheral blood leucocyte in septic rats

Binding capacity of GR in the peripheral white blood cells

The binding capacities of GR in the peripheral white blood cells were significantly decreased in a time dependent manner in the sepsis groups in comparison to the control groups ($t = 23.045$, $P < 0.01$). After rhubarb administration, the binding capacities of GR in peripheral white blood cells were significantly increased compared with that in the sepsis groups in a time dependent manner, however, the binding capacities of GR in peripheral white blood cells were still remarkably decreased in comparison to that in the control groups ($t = 19.965$, $P < 0.05$) [Figure 1A].

Binding activity of GR in the liver cytosol

The binding activities of GR in the liver cytosol were significantly decreased in a time dependent manner in the sepsis groups in comparison to that in the control groups ($t = 24.395$, $P < 0.05$). After rhubarb administration, the binding capacities of GR in the liver cytosol were significantly increased compared with that in the sepsis groups at all time points ($t = -6.486$, $P < 0.05$). However, the binding capacities of GR in peripheral white blood cells were still remarkably decreased in comparison to that in the control groups ($t = 17.140$, $P < 0.05$) [Figure 1B].

Effects of rhubarb on the percentages of CD4$^+$ T cells in the peripheral blood of septic rats

The percentages of CD4$^+$ T cell in peripheral blood were significantly increased at 12 h in the sepsis group compared with that at 12 h in the control group ($t = -3.395$, $P < 0.01$). Moreover, the CD4$^+$ T cell percentage was significantly lower at 24 h than at 72 and 12 h subgroups of sepsis groups (compared with 72 h, $t = 5.397$, $P < 0.05$, *vs.* 12 h, $t = 4.513$, $P < 0.05$). Furthermore, the CD4$^+$ T cell percentage were significantly lower after rhubarb treatment for 12 h compared to that at the same time point of sepsis groups ($t = 3.325$, $P < 0.05$) [Figure 2].

Effects of rhubarb on the percentages of CD4$^+$CD25$^+$ T cells in the peripheral blood of septic rats

No significant differences of CD4$^+$CD25$^+$ T cell percentages were observed in the peripheral blood at different time points between control and sepsis groups ($F = 0.925$, $P = 0.432$). The percentage of CD4$^+$CD25$^+$ T cell at 12 h subgroup in the rhubarb group was significantly higher than that in the control and sepsis groups ($t = -21.063$, $P < 0.01$; $t = -24.521$, $P < 0.01$, respectively); however,

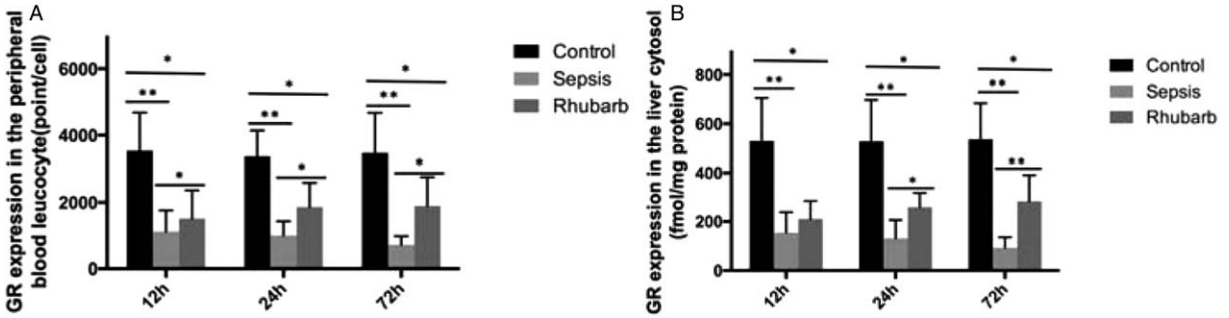

Figure 1: GR expression in the liver cytosol (A) and peripheral blood leucocyte (B) of rats in each subgroups. The binding activity of GR in liver cytosol was detected by the improved radiation ligands binding assay, and the binding capacity of GR in peripheral blood leucocyte was detected by improved method. The binding capacities of GR in the peripheral white blood cells and binding activities of GR in the liver cytosol were significantly decreased in a time dependent manner in the sepsis groups in comparison to the control groups ($^*P < 0.05$). After rhubarb administration, both were significantly increased compared with that in the sepsis groups at all time points ($^{**}P < 0.05$). GR: glucocorticoids receptor.

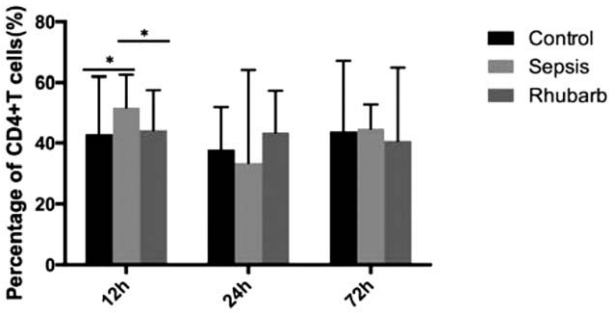

Figure 2: Percentage of $CD4^+$ T cells in the peripheral blood of rats in each subgroup. The number of $CD4^+$ T cells in peripheral blood was counted by flow cytometer, and then percentages of $CD4^+$ T cells were calculated. The percentages of $CD4^+$T cell in peripheral blood were significantly increased at 12 h in the sepsis group compared with that at 12 h in the control group and decreased dramatically after rhubarb treatment ($^*P < 0.05$). Moreover, the $CD4^+$ T cell percentage was significantly lowest at 24 h ($^*P < 0.05$).

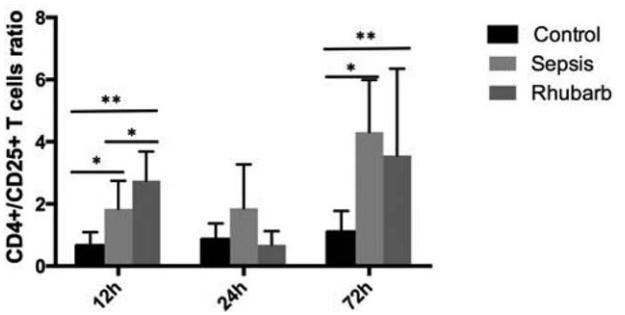

Figure 4: Ratio of $CD4^+/CD25^+$ T cells in the peripheral blood of rats in each subgroup. The ratios of $CD4^+/CD25^+$ T cell in the peripheral blood were significantly increased at 12 h in the rhubarb group in comparison to that at 12 h in both sepsis and control group (*vs.* control group, $^{**}P < 0.01$; *vs.* sepsis group, $^*P < 0.05$). However, there were no significant differences between three groups at different time points ($P > 0.05$). The percentages of $CD25^+$ T cell were significantly higher at 72 h in the sepsis group than that at 72 h both in the control group ($^*P < 0.05$).

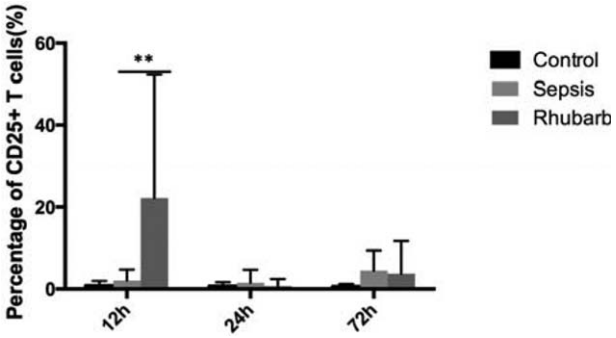

Figure 3: Percentage of $CD4^+CD25^+$ T cells in the peripheral blood of rats in each subgroup. No significant differences of $CD4^+CD25^+$ T cell percentages were observed in the peripheral blood at different time points between control and sepsis groups ($P > 0.05$). The percentage of $CD25^+$ T cell at 12 h subgroup in the rhubarb group was significantly higher than that in the control and sepsis groups ($^{**}P < 0.05$), however the percentage of $CD4^+CD25^+$ T cell decreased dramatically at 24 h in the sepsis group.

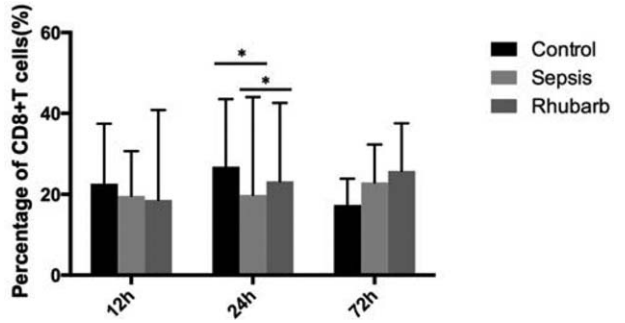

Figure 5: Percentage of $CD8^+$ T cells in the peripheral blood of rats in each subgroup. The percentages of $CD8^+$ T cell in the peripheral blood were significantly decreased at 24 h in the sepsis group compared with that at 24 h in the control group ($^*P < 0.05$), but were significantly increased after 24 h rhubarb treatment compared with that in the sepsis group ($^*P < 0.05$). No significant differences were observed between three groups at 12 and 72 h time points ($P > 0.05$).

the percentage of $CD25^+$ T cell decreased dramatically at 24 h in the sepsis group [Figure 3].

Effects of rhubarb on the ratio of $CD4^+/CD25^+$ T cells in the peripheral blood of septic rats

The ratio of $CD4^+/CD25^+$ T cell in the peripheral blood were significantly increased at 12 h in the sepsis group in comparison to that at 12 h in the control group ($t = 2.568$, $P < 0.05$), moreover the percentages of $CD4^+/CD25^+$ T cell in the peripheral blood were significantly increased in the rhubarb group compared with that both in the control and sepsis groups at the same time point, respectively (compared with control group, $t = 3.064$, $P < 0.01$; *vs.* sepsis group, $t = 2.649$, $P < 0.05$). However, there were no significant differences between control, sepsis and rhubarb groups at different time points ($P > 0.05$). The ratio of $CD4^+/CD25^+$ T cell were significantly higher at 72 h in the sepsis group than that at 72 h both in the control group ($t = 2.508$, $P < 0.05$). The ratio of $CD4^+/CD25^+$ T cell at 72 h subgroups were significantly higher than that at 24 and 12 h subgroups in the rhubarb group ($t = 3.378$, $P < 0.05$) [Figure 4].

Effects of rhubarb on the percentages of $CD8^+$ T cells in the peripheral blood of septic rats

The percentages of $CD8^+$ T cell in the peripheral blood were significantly decreased at 24 h in the sepsis group compared with that at 24 h in the control group ($t = -2.191$, $P < 0.05$), but were significantly increased after 24 h rhubarb treatment compared with that in the sepsis group ($t = 2.953$, $P = 0.026$). No significant differences were observed between control, sepsis, and rhubarb groups at 12 and 72 h time points ($P > 0.05$, respectively) [Figure 5].

Effects of rhubarb on the ratio of $CD8^+/CD25^+$ T cells in the peripheral blood of septic rats

There were no significant differences of $CD8^+/CD25^+$ T cells ratio between the sepsis groups and the control groups at all time points. The ratio of $CD8^+/CD25^+$ T cells was significantly increased at 12 h in the rhubarb group compared with that at 12 h both in the control and sepsis groups ($t = 2.191$, $P < 0.05$), and was decreased at 24 and 72 h in the rhubarb group. No significant differences of $CD8^+/CD25^+$ T cell ratio was showed between the sepsis groups and the rhubarb groups at 24 and 72 h time point ($t = 3.389$, $P > 0.05$) [Figure 6].

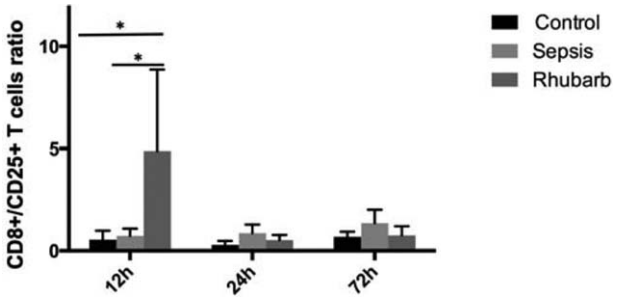

Figure 6: Ratio of $CD8^+/CD25^+$ T cells in the peripheral blood of rats in each subgroup. There were no significant differences of $CD8^+/CD25^+$ T cells ratio between the sepsis groups and the control groups at all time points. The ratio of $CD8^+/CD25^+$ T cells was significantly increased at 12 h in the rhubarb group compared with that at 12 h both in the control and sepsis groups ($^*P < 0.05$), and was decreased at 24 and 72 h in the rhubarb group ($^*P < 0.05$).

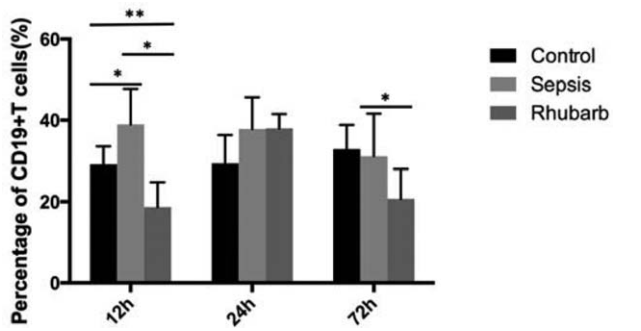

Figure 7: Percentage of $CD19^+$ B cells in the peripheral blood of rats in each subgroup. The percentages of $CD19^+$ T cell in peripheral blood were significantly increased at 12 h in the sepsis group in comparison to that in the control group and 72 h sepsis group ($^*P < 0.05$). Significant decreased percentage of $CD19^+$ B cell at both 12 and 72 h in the rhubarb group was observed compared with that at 24 h in the sepsis group ($^{**}P < 0.05$). However, there was no significant difference was showed at 24 h between control, sepsis, and rhubarb groups ($P > 0.05$).

Effects of rhubarb on the percentage of $CD19^+$ B cells in the peripheral blood of septic rats

The percentages of $CD19^+$ B cell in peripheral blood were significantly increased at 12 h in the sepsis group in comparison to that in the control group ($t = 2.993$, $P < 0.05$), and was significantly higher at 12 h than 72 h in the sepsis group ($t = 2.041$, $P < 0.05$). Significant decreased percentage of $CD19^+$ B cell at both 12 and 72 h in the rhubarb group was observed compared with that at 24 h in the sepsis group ($t = -2.139$, $P < 0.05$). However, there was no significant difference showed at 24 h between control, sepsis, and rhubarb groups ($P > 0.05$) [Figure 7].

Effects of rhubarb on the percentage of natural killer cells in the peripheral blood of septic rats

The percentage of NK cells in the peripheral blood was significantly decreased at 12 h both in the sepsis group and rhubarb group compared with that at 12 h in the control group ($t = -2.022$, $P < 0.05$). The percentage of NK cells was lower at 24 and 72 h in the sepsis group than that in the control groups and rhubarb groups, but no significant differences were observed ($P > 0.05$). The percentage of NK cells at 24 h was significantly increased than that at 12 and 72 h in the rhubarb groups ($t = 2.973$, $P < 0.05$; $t = 2.562$, $P < 0.05$, respectively) [Figure 8].

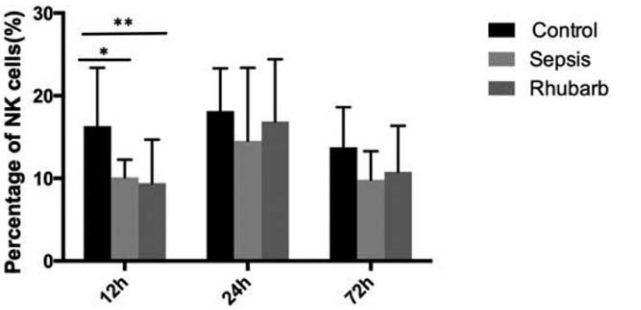

Figure 8: Percentage of NK cells in the peripheral blood of rats in each subgroups. The percentage of NK cells in the peripheral blood was significantly decreased at 12 h both in the sepsis group and rhubarb group compared with that at 12 h in the control group ($^*P < 0.05$). The percentage of NK cells was lower at 24 and 72 h in the sepsis group than that in the control and rhubarb groups, but no significant differences were observed ($P > 0.05$). The percentage of NK cells at 24 h was significantly increased than that at 12 and 72 h in the rhubarb groups ($^{**}P < 0.05$). NK: natural killer.

Discussion

In our study, we found the binding activity of GR in liver cytosol and binding capacity of GR in peripheral blood leucocyte were significantly decreased in a time-dependent manner in sepsis group and were increased in a time-dependent manner after rhubarb administration. The percentages of $CD19^+$ B cell in rats' peripheral blood were significantly increased at 12 h in the sepsis group, while the percentages of NK cell were significantly decreased. Moreover, the percentages of $CD4^+$ T cell in rats' peripheral blood were significantly decreased at 24 h in the sepsis group, while the percentages of $CD8^+$ T cell were significantly increased. The decreased expression of $CD4^+$ T cells and NK cells may inhibit by the increased expression of $CD8^+$ and $CD19^+$ B cells. Our study also suggested that the percentages of $CD4^+$ $CD25^+$Tr cells in rats' peripheral blood were significantly increased at 12 h in the sepsis group.

It has been confirmed that GR is widely expressed by the mammals nucleated cells, extremely in hepatocytes. Activation of GR could remarkably inhibit pro-inflammatory cytokines and prevent the occurrence of multiple organs failure (MOF).[10] However, GR blockade may cause the increased permeability of vascular walls and accelerate the process of MOF.[11] It was indicated that GR expression may involve in the process of systemic inflammatory response syndrome (SIRS) and even MOF induced by burning injury. It was indicated the binding activity of GR in liver cytosol and the binding capacity of GR in peripheral blood leucocyte of burn septic rats were significantly decreased in a time dependent manner.[12-14] In our present study, the binding activity of GR in liver cytosol and the binding capacity of GR in peripheral blood leucocyte were increased in a time dependent manner after rhubarb administration. Previous studies have shown that CD19 could constantly be expressed during the activation of B cells, the expression levels of CD19 may related with expression level of B cells in blood.[15] Not like T cells and B cells, NK cells belong to a group of large granular

lymphocytes.[16] CD25 are mainly expressed in the mature activated T lymphocytes, and the expression level of $CD25^+$ could directly indicate the extent of T lymphocytes activation.[17,18] However, $CD8^+$ T cells could inhibit the activations and proliferations of $CD4^+$ T cells through the cell to cell dependent attachments.[19] Our results show that the percentages of $CD4^+$ $CD25^+$ Tr cells in rats' peripheral blood were significantly increased at 12 h; the percentages of $CD4^+$ T cells in rats' peripheral blood were also significantly increased at 24 h in the rhubarb group in comparison to the sepsis group. While the percentages of $CD19^+$ B cells in rats' peripheral blood were significantly decreased at 72 h in the rhubarb group in comparison to the sepsis group; and the percentages of NK cells in rats' peripheral blood were remarkably increased in the rhubarb group in comparison to the control group.

Recently, there are growing concerns about the anti-inflammatory and immunoregulatory effects of rhubarb. The protective effects of rhubarb on the anti-inflammation and immunity regulation provide a new method for the treatment of sepsis. However, the present study exists some limitations: the survival rates of sepsis and rhubarb groups were not established; the number of rats in each group was not completely enough; the immunity system contained innate immunity and adaptive immunity, only partial indicators of innate immunity were detected in the present study.

The morbidity and mortality of sepsis are extremely high in ICU, and the immune response plays an important role in the occurrence and progression of sepsis. Our previous studies indicated that the rhubarb could reduce the expression and concentration of TNF-α in plasma and liver, thus further inhibit the severity of inflammation.[20] In our current study, the effect of rhubarb on immune response in sepsis rat model in *in vivo* experiments was observed. However, the relationship between GR expression and cellular immunity dysfunctions and the mechanism of rhubarb regulating GR expression in cell immunity regulation need further explored.

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Conflicts of interest

None.

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