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Original Article

Effects of Shenfu Decoction on Neutrophil Chemotactic Function in Septic Mice

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Abstract

Background and objectives: Sepsis involves a complex cascade of inflammatory reactions and immune system dysregulation. Neutrophils play a crucial role in modulating the anti-inflammatory response, which is vital for managing sepsis. Impaired chemotaxis of granulocytes can significantly impact the outcome of sepsis. Shenfu Decoction, by tonifying *Qi* and warming Yang, enhances the propelling function of *Qi* for promoting the chemotactic function of neutrophils. This study aimed to investigate the effects of Shenfu Decoction on the chemotactic function of neutrophils in septic mice and the underlying mechanisms.

Methods: Thirty 10-week-old specific-pathogen-free male C57BL/6J mice were randomly divided into five groups: sham operation, model, and low-, medium-, and high-dose Shenfu Decoction treatment groups (n = 6 in each group). Sepsis was induced using cecum ligation and puncture procedures. The sham-operated group served as the control. The drug was administered 6 h after surgery; the sham-operated and model groups received saline, while the treatment groups were gavaged every 12 h with the respective concentrations of Shenfu Decoction. Four hours after the last gavage, the mice were euthanized, and samples were collected to determine neutrophil counts and related indices. Primary neutrophils were extracted from the peripheral blood of septic mice and divided into blank control, sham-operated, low-dose, and high-dose groups. These cells were cultured with serum containing the respective treatments to measure neutrophil chemotactic distance, intracellular calcium ion concentration, and the expression levels of chemokine receptors and P2X1 receptors.

Results: Compared with the sham-operated group, the total number of colonies and the number of neutrophils in the peritoneal lavage fluid were increased in the model group ($P < 0.05$). In the treatment groups, the number of neutrophils in the peritoneal lavage fluid was significantly increased ($P < 0.05$), while the number of neutrophils in the blood was decreased. Compared with the blank control group, the neutrophil chemotaxis distance was significantly prolonged in the sham-operated group. Additionally, the expression levels of P2X1 and FPR1 receptors were decreased, the expression levels of CXCR1 and CXCR2 receptors were increased ($P < 0.05$), and the calcium ion concentration was decreased ($P > 0.05$). Compared with the sham-operated group, the treatment groups exhibited a prolonged neutrophil chemotaxis distance, significantly decreased expression levels of P2X1 and FPR1 receptors, significantly increased expression levels of CXCR1 and CXCR2 receptors ($P < 0.05$), and significantly decreased calcium ion concentrations ($P < 0.05$). These effects were positively correlated with the Shenfu Decoction dosage.

Conclusions: Shenfu Decoction can improve the chemotactic function of neutrophils, possibly through the downregulation of P2X1 receptor expression. Its effects are positively correlated with the dosage.

Keywords: Shenfu Decoction; Sepsis; Neutrophils; Chemotactic function; Immunomodulation; Inflammatory response.

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Introduction

Sepsis is a systemic cascade of inflammatory responses and immune dysregulation caused by severe infection, which can lead to multi-organ failure and death.[1,2] Studies have shown that the incidence and mortality rates of sepsis worldwide remain extremely high, with 33.6% of critically ill patients in China diagnosed with sepsis and an overall mortality rate of 29.0% among those with sepsis. It is reported that there are more than 19 million sepsis patients globally each year, with six million deaths, resulting in a mortality rate of over one-quarter. Septic shock can lead to a mortality rate of up to 60%.[3,4] Current treatments are unable to address all aspects of sepsis. In recent years, research has focused on enhancing anti-inflammatory treatment, strengthening immune regulation, and improving patients' ability to clear pathogens. Neutrophils play a crucial role in the cellular immune response during sepsis. Studies have indicated that under the stimulation of bacterial toxins and cytokines during sepsis, neutrophils become dysfunctional, their chemotactic ability toward infection sites is weakened, and their capacity to clear pathogens is reduced. Shenfu Decoction, a commonly used formula for reinforcing *Qi* and consolidating collapse, has effects such as enhancing the body's non-specific immune function and is widely used in clinical emergencies with significant efficacy.[5] It is generally believed that sepsis is a syndrome of excess, dominated by heat toxins, while septic shock is a syndrome of deficiency. Improper application of Shenfu Decoction in clinical practice can lead to errors in distinguishing between deficiency and excess, resulting in missed opportunities for treatment. Guided by the principles in "*Huangdi Neijing*" (The Yellow Emperor's Canon of Medicine) that "where pathogens gather, there must be deficiency" and "pathogenic factors cannot harm humans alone," we consider that deficiency may be present throughout the pathogenesis of critical illnesses. Shenfu Decoction can support the body's immunity from the perspective of reinforcing healthy *Qi*, enabling it to combat pathogenic *Qi* effectively. Shenfu Decoction is commonly used in the emergency treatment of sepsis in clinical practice and has achieved good clinical outcomes. To clarify the indications and efficacy of Shenfu Decoction in sepsis, which is generally considered a syndrome of excess and heat in its literal interpretation, we started from the perspective of neutrophil chemotactic function to explore the effects of Shenfu Decoction on septic mice and its possible mechanisms.

Materials and methods

Experimental animals and grouping

Thirty 10-week-old specific-pathogen-free male C57BL/6J mice, weighing 20 ± 2 g, with an animal experiment qualification certificate: SCXL(Hu)2020-0018, were divided into five groups using a random number table method (n = 6 in each group): sham operation, septic model, model + low-dose Shenfu Decoction, model + medium-dose Shenfu Decoction, and model + high-dose Shenfu Decoction.

Composition and preparation of the medicine

The composition of Shenfu Decoction includes raw sun-dried ginseng and processed aconite root in a 3:1 ratio (390 g and 130 g, respectively). The herbal pieces were purchased from the Pharmacy Department of Wuhan Traditional Chinese Medicine Hospital. First, the processed aconite root was soaked in 4,000 mL of water for 1 h, brought to a boil over high heat, and then simmered on low heat for 1 h. Next, ginseng was added, brought to a boil again over high heat, and then simmered on low heat for another hour. Afterward, the decoction was filtered through sterile gauze to collect 3,000 mL of liquid. Additional water was added to the residue, boiled again, and a second batch of 3,000 mL of liquid was collected, resulting in a total of 6,000 mL of decoction. The concentration of the medicine was 52 mg/0.6 mL. Based on the literature and equivalent dose calculations for experimental animals,[6] a 20 g mouse required doses of 52 mg, 104 mg, and 208 mg, equivalent to a 70 kg adult human daily dosage of 20 g, 40 g, and 80 g, respectively. Considering the stomach capacity of mice, the plan was to administer 0.6 mL of the decoction twice daily. Therefore, the decoction was concentrated by heating to prepare low, medium, and high concentrations (52 mg/0.6 mL, 104 mg/0.6 mL, 208 mg/0.6 mL) and stored at 4°C in the refrigerator.

Main reagents and instruments

Main reagents

The BCA Protein Assay Kit (Product No. AS1086) was purchased from ASPEN. The Mouse P2X1 ELISA Kit (Product No. ELK6575), Mouse FPR1 ELISA Kit (Product No. ELK4832), Mouse CXCR1 ELISA Kit (Product No. ELK2893), and Mouse CXCR2 ELISA Kit (Product No. ELK7031) were acquired from ELK Biotechnology. The primary antibodies (fMLP and IL-8), Product Nos. HY-P0224 and HY-P81106) and secondary antibodies (Product No. HY-P81016) were purchased from MCE. Fetal Bovine Serum (Product No. 141215) was sourced from Hangzhou Tianhang Biotechnology Co., Ltd. The Calcein AM Assay Kit was purchased from Shanghai Yisheng Biotechnology Co., Ltd. (Catalog No. 40719ES50).

Major instruments

The instruments used included an Inverted Microscope (Olympus Corporation, Japan, IX51), Biological Microscope (Nikon, Japan, YS5100), Imaging System (Q-IMAGING, USA, MicroPublisher), DR-200Bs Microplate Reader (Diatek Instruments), GNP9160 Constant Temperature Incubator (Shanghai Jinghong Experimental Equipment Co., Ltd.), DYY-6C Electrophoresis Apparatus (Beijing Liuyi Instrument Factory), RM2016 Microtome (Leica Instruments Shanghai Ltd.), and JT-12K Dehydrator and JB-P5 Embedding Machine (Wuhan Jujie Electronics Co., Ltd.).

Model establishment

This experiment was approved and recorded by the Ethics Committee of Wuhan Traditional Chinese Medicine Hospital. The septic model was prepared using the cecal ligation and puncture method. Mice were intraperitoneally injected with 10% chloral hydrate at a dose proportional to body weight (0.1 mL/10 g). Two to three minutes after the anesthetic effect appeared, an incision was made from the xiphoid process towards the tail, exposing and ligating half the length of the cecum. A 5 mL syringe needle was used to puncture the ligated section of the cecum once, which was then slightly squeezed before being returned, and the abdomen was closed. The sham operation group underwent a sham operation where, after anesthesia, an incision was made along the midline near the xiphoid process, the cecum was exposed and then immediately returned without ligation, puncture, or squeezing. After surgery, all groups of mice had free access to water and food.

Administration method

In accordance with the principles and guidelines presented in the "Methodology of Pharmacological Research on Traditional Chi-

Table 1. Comparison of the number of neutrophils in the peritoneal lavage fluid and blood among different groups of mice (×10^9/L, $\bar{x} \pm S$)

Groups	n	Number of neutrophils	
		Peritoneal lavage fluid	Blood
Sham operation	6	0.01 ± 0.01	0.85 ± 0.06
Septic model	6	0.13 ± 0.02**	1.91 ± 0.03**
Low dose of Shenfu Deoction	6	0.16 ± 0.02△	1.81 ± 0.06
Medium dose of Shenfu Deoction	6	0.21 ± 0.01△△	1.36 ± 0.07△△
High dose of Shenfu Deoction	6	0.23 ± 0.01△△	1.18 ± 0.08△△

vs Sham operation group, *P < 0.05,**P < 0.01;vs Septic model group, △P < 0.05,△△P < 0.01.

nese Medicine",[6] the equivalent dose was calculated based on the conversion of the body surface area ratio of animals. Mice in the Shenfu Decoction groups were administered 0.03 mL/g, totaling 0.6 mL, divided into two doses (at a 12-h interval) via gavage after 6 h of cecal ligation and puncture surgery, for three consecutive days. The sham operation and model groups received an equal volume of saline via gavage for three days, once every 12 h. Food and water were withheld 2 h before gavage, and experimental specimens were collected 4 h after the last gavage.

Neutrophil isolation and culture

Primary neutrophils were extracted from the peripheral blood of septic mice using the suspension cell separation method. Through drug serum gradient screening, the optimal drug concentration was determined (10% drug-containing serum intervention for 24 h), and the cells were cultured with drug-containing serum (10%) for five days before testing. The blank control group was cultured with fetal bovine serum, the sham operation group with serum from sham-operated mice, the low-dose group with drug-containing serum at a low dose of Shenfu Decoction, and the high-dose group with drug-containing serum at a high dose of Shenfu Decoction.

Specimen testing

Number of neutrophils

The number of neutrophils in peritoneal lavage fluid and blood was measured using an automatic hematology analyzer. The chemotactic distance of neutrophils was assessed through agarose chemotaxis experiments. The expression levels of chemotactic molecular receptors and P2X1 receptors in neutrophils were detected by Western Blot. Calcein staining was conducted to measure calcium ion concentration.

Agarose chemotaxis assay

Agarose chemotaxis assay for measuring neutrophil chemotactic distance: Take a 50 mL centrifuge tube, add 0.45 g of agarose powder to 30 mL of ultrapure water, and mix gently by slight shaking. Heat the mixture in a microwave until boiling, remove and shake for 5 s, then microwave for 20 s. Repeat this process several times until the agarose is completely dissolved. Quickly transfer 3 mL of the dissolved agarose into a 35 mm petri dish and allow it to cool slowly to room temperature, avoiding the formation of bubbles. Once solidified, the agarose gel is formed. Use a hollow tube with a diameter of 3.5 mm to punch two holes in the gel, spaced 2.4mm apart. Carefully remove the agarose inside the holes using a vacuum aspirator. Place the petri dish in a 37°C incubator for 1 h to allow temperature equilibration. Use the vacuum aspirator again to carefully remove any excess liquid from the holes, preparing them for use. Add 5 μL of chemoattractant (fMLP as the terminal chemotactic molecule and IL-8 as the intermediate chemotactic molecule) to one hole and 5 μL of cell suspension to the other hole. After incubation in a 37°C incubator for 2 h, observe and photograph the chemotactic distance of the central granulocytes under an inverted microscope.

Statistical analysis

SPSS 18.0 statistical software was used for data analysis. Quantitative data, if normally distributed, are presented as mean ± standard deviation ($\bar{x} \pm S$). The independent samples t-test was used for comparisons between two groups, while analysis of variance was used for comparisons among multiple groups. A P-value of less than 0.05 was considered statistically significant.

Results

The effect of Shenfu Decoction on the number of neutrophils in peritoneal lavage fluid and peripheral blood of mice

Compared with the sham operation group, the number of neutrophils in both the peritoneal lavage fluid and blood of mice in the sepsis model group increased significantly ($P < 0.05$). This indicates that the sepsis model was successfully established and that the number of neutrophils in the body increases during sepsis, migrating toward the site of infection. Compared with the model group, the number of neutrophils in the peritoneal lavage fluid of the various dosage groups treated with Shenfu Decoction increased. This increase was dose-dependent, with a *statistically significant difference* ($P < 0.05$). This suggests that Shenfu Decoction mobilized neutrophils throughout the body to migrate to the peritoneal cavity. Meanwhile, the number of neutrophils in the blood of the various dosage groups treated with Shenfu Decoction decreased. The low-dose group showed no *statistical difference* ($P > 0.05$), while the medium and high-dose groups showed a *statistical difference* ($P < 0.05$). This indicates that Shenfu Decoction effectively mobilized neutrophils from the blood to the peritoneal cavity while also enhancing the body's production of neutrophils in a dose-dependent manner (Table 1 and Fig. 1).

The effect of Shenfu Decoction on the chemotaxis distance of neutrophils

In the presence of different chemotactic agents, compared with the blank control group, the chemotaxis distance of neutrophils in the sham operation group was significantly extended, showing a statistically significant difference ($P < 0.01$). Compared with the sham operation group, the chemotaxis distance of neutrophils in the Shenfu Decoction group was further extended, with a statistically

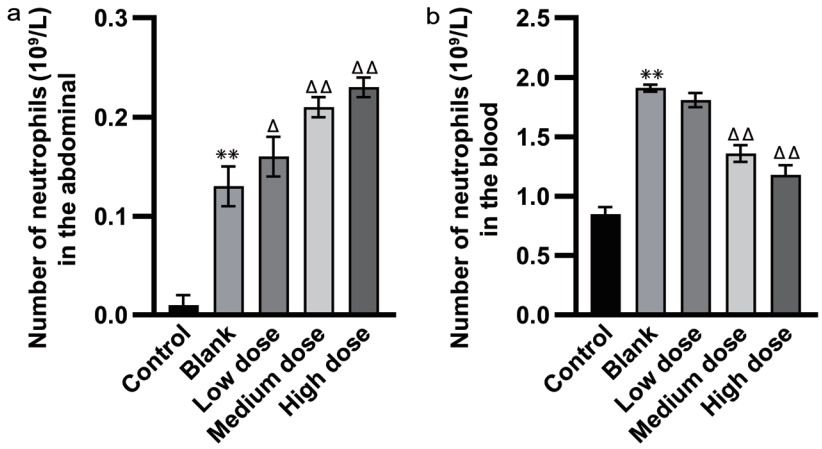

Fig. 1. Comparison of the number of neutrophils in the peritoneal lavage fluid and blood among different groups of mice. (a) Neutrophil count in the abdominal cavity; (b) Neutrophil count in the blood.

Table 2. The effect of Shenfu Decoction on the chemotaxis distance of neutrophils under different chemotactic agent conditions (μm, $\bar{x} \pm S$)

Groups	n	fMLP	IL-8
Blank control	6	424.87 ± 19.63	711.85 ± 20.25
Sham operation	6	1,071.39 ± 21.92**	936.17 ± 23.12**
Low dose of Shenfu Decoction	6	1,298.56 ± 24.15$^{\triangle}$	1,074.62 ± 28.76$^{\triangle}$
High dose of Shenfu Decoction	6	1,862.25 ± 36.34$^{\triangle\triangle}$	1,885.25 ± 31.74$^{\triangle\triangle}$

vs Blank control group, *P < 0.05,**P < 0.01; vs Sham operation group, $^{\triangle}P$ < 0.05,$^{\triangle\triangle}P$ < 0.01. fMLP, formyl-methionine-leucine-phenylalanine; IL, interleukin.

significant difference (P < 0.05). The effect was stronger in the high-dose group than in the low-dose group. The chemotactic factor IL-8 had a weaker effect on neutrophil chemotaxis compared to fMLP (Table 2 and Fig. 2).

The effect of Shenfu Decoction on the expression of chemokine receptors and P2X1 receptors

Compared with the blank control group, the expression levels of P2X1 and FPR1 receptors in the sham operation group decreased, while the expression levels of CXCR1 and CXCR2 receptors increased, showing statistical differences (P < 0.05). Compared with the sham operation group, the Shenfu Decoction group exhibited a significant decrease in the expression levels of P2X1 and FPR1 receptors and a significant increase in the expression levels of CXCR1 and CXCR2 receptors, with statistical differences (P < 0.01) (Table 3 and Fig. 3).

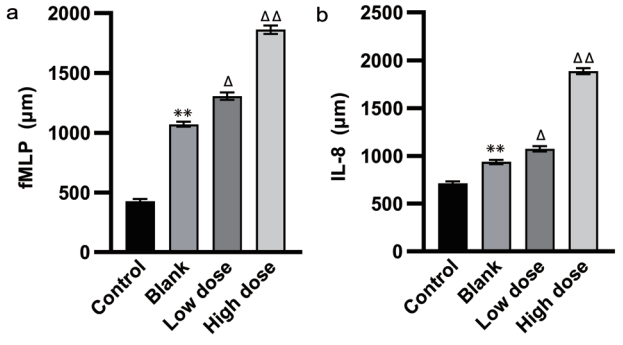

Fig. 2. The effect of Shenfu Decoction on the chemotaxis distance of neutrophils under different chemotactic agent conditions. (a) fMLP; (b) IL-8. vs Blank control group, *P < 0.05,**P < 0.01; vs Sham operation group, $^{\triangle}P$ < 0.05,$^{\triangle\triangle}P$ < 0.01. fMLP, formyl-methionine-leucine-phenylalanine; IL, interleukin.

Changes in the intracellular calcium ion concentration in neutrophils

Figure 4 show that, compared with the blank control group, the concentration of calcium ions within neutrophils in the sham operation group decreased. Compared with the sham operation group, the concentration of calcium ions within neutrophils in the Shenfu Decoction group significantly decreased.

Discussion

The main pathogenesis of sepsis involves infection-induced immunodysregulation, systemic cascading inflammatory responses, and coagulation dysfunction, leading to multi-organ failure and even death by shock.[7] The causes of sepsis may include various assaults, such as trauma and infections, where bacteria and toxins enter the body, triggering the production of inflammatory mediators that activate the body's inflammatory response. An exacerbated inflammatory response increases immune suppression, thereby worsening the inflammation and further damaging the body. Typical symptoms include chills, fever, increased heart rate, shortness of breath, altered mental status, and edema, which may accompany organ dysfunction (cardiovascular, lung, brain, kidney), inadequate tissue perfusion, hypotension, lactic acidosis, oliguria, and acute changes in consciousness. Septic shock presents symptoms of sepsis along with hypotension, consciousness disturbances, a

Table 3. Comparison of the relative expression levels of chemokine receptors and P2X1 receptors in neutrophils among different groups (n = 6, $\bar{x} \pm S$)

Groups	P2X1	FPR1	CXCR1	CXCR2
Blank Control	0.51 ± 0.12	0.33 ± 0.03	0.06 ± 0.01	0.16 ± 0.02
Sham Operation	0.36 ± 0.14**	0.29 ± 0.08*	0.08 ± 0.02*	0.20 ± 0.07*
Low dose of Shenfu Decoction	0.19 ± 0.03△△	0.12 ± 0.04△△	0.13 ± 0.06△△	0.48 ± 0.24△△
High dose of Shenfu Decoction	0.07 ± 0.01△△	0.04 ± 0.02△△	0.31 ± 0.15△△	0.65 ± 0.19△△

vs Blank control group, *P < 0.05,**P < 0.01; vs Sham operation group, △P < 0.05,△△P < 0.01. CXCR1, C-X-C chemokine receptor type 1; CXCR2, C-X-C chemokine receptor type 2; FPR1, formyl peptide receptor 1; P2X1, purinergic receptor P2X ligand - gated ion channel 1.

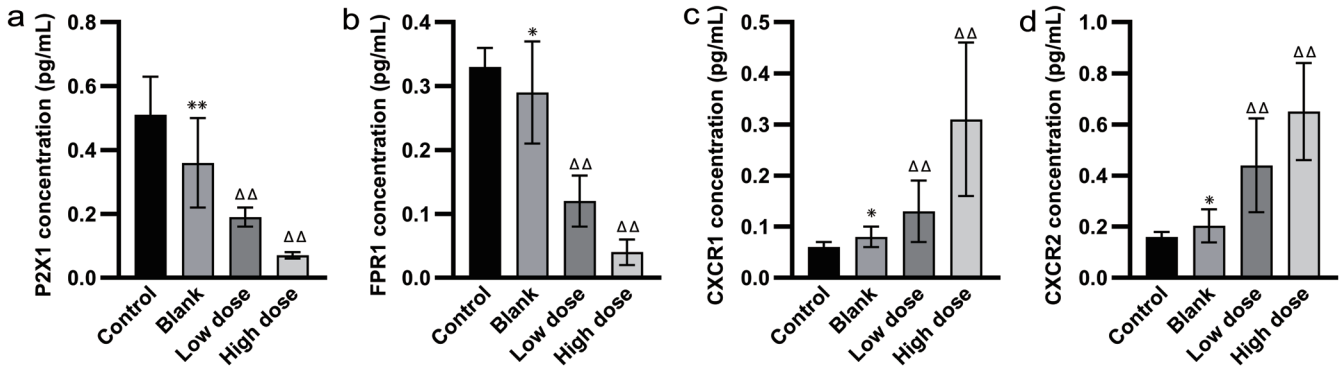

Fig. 3. Comparison of the relative expression levels of chemokine receptors and P2X1 receptors in neutrophils among different groups. (a) Expression levels of P2X1 receptors; (b) Expression levels of FPR1 receptors; (c) Expression levels of CXCR1 receptors; (d) Expression levels of CXCR2 receptors. vs Blank control group, *P < 0.05,**P < 0.01; vs Sham operation group, △P < 0.05,△△P < 0.01. CXCR1, C-X-C chemokine receptor type 1; CXCR2, C-X-C chemokine receptor type 2; FPR1, formyl peptide receptor 1; P2X1, purinergic receptor P2X ligand - gated ion channel 1.

rapid and weak pulse, cold and damp limbs, and oliguria or anuria.

Based on the above manifestations, sepsis in Traditional Chinese Medicine (TCM) is categorized under the conditions of "Shang Han" (Cold Damage), "Wen Bing" (Warm Diseases), and "Jue Tuo" (Collapsing Syndrome), among others. In the early stage, it is categorized as "Wen Bing" and "Re Zheng" (Heat Syndromes), gradually evolving into "Tuo Zheng" (Collapse Syndrome).[8] The etiology includes external factors such as the Six Excesses (wind, cold, summer heat, dampness, dryness, and fire) and internal factors such as the Seven Emotions (joy, anger, worry, contemplation, sorrow, fear, and startlement), as well as injuries that do not fall under either internal or external categories, with pathological products such as heat toxins, blood stasis, and phlegm turbidity. Some scholars suggest that sepsis, characterized by symptoms such as chills, fever, palpitations, and shortness of breath, is primarily caused by toxic heat, and recommend methods to clear heat and detoxify.[9] Although this approach has certain effects, it cannot prevent the deterioration of sepsis or its transition to septic shock.

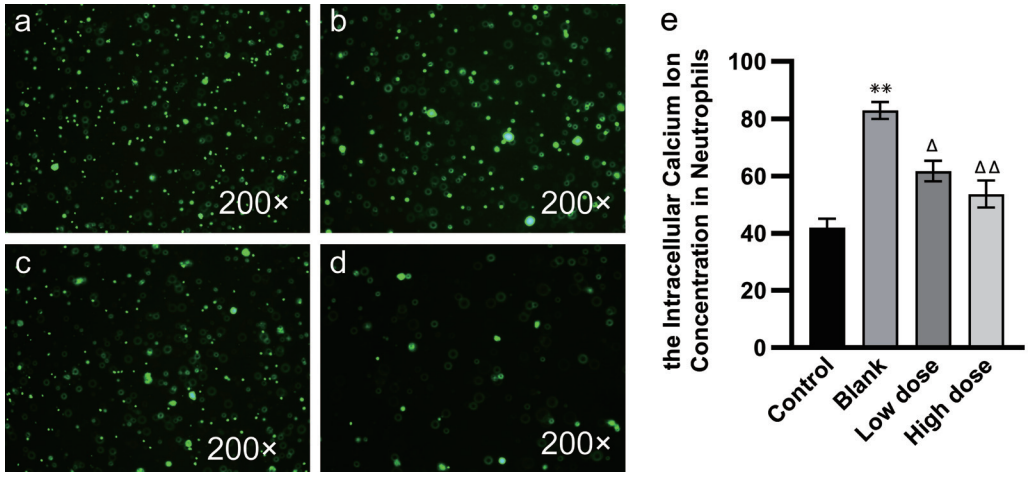

Fig. 4. Calcium ion concentration in neutrophils of each group. (a) Blank control; (b) Sham operation; (c) Low dose of Shenfu Decoction; (d) High dose of Shenfu Decoction; (e) Changes in the intracellular calcium ion concentration in neutrophils. vs Blank control group, *P < 0.05,**P < 0.01; vs Sham operation group, △P < 0.05,△△P < 0.01.

The "*Huangdi Neijing*" states, "When the righteous *Qi* resides within the body, pathogenic factors cannot interfere," and "Pathogenic factors cannot harm the body alone," highlighting the crucial role of the body's righteous *Qi* in the development of sepsis. The modern understanding of sepsis places significant emphasis on immune function, precisely the righteous *Qi* mentioned in the "*Huangdi Neijing*". Through years of clinical practice by generations of TCM practitioners at our institution, we have identified that a deficiency in righteous *Qi* is the fundamental cause of sepsis, affecting the entire course of the disease. An abundance of righteous *Qi* can prevent infection in the early stages, avert the progression of disease and expulsion of pathogens in the mid-stage, promote recovery, and prevent sequelae in the later stages.[10] As stated in the "*Huangdi Neijing*", "Where pathogens gather, there must be a deficiency." The onset of sepsis indicates that the external defensive barrier has been compromised and the protective *Qi* has been breached. In clinical practice, we choose Shenfu Decoction to enhance *Qi*, warm *Yang*, secure the collapse, and support the righteous *Qi* while expelling pathogens, achieving favorable therapeutic outcomes. Research shows that Shenfu Decoction improves systemic circulation and microcirculation, protects organ cells, and enhances cellular immunity.[11] In real clinical conditions, especially in critical cases without antibiotic sensitivity results, the safety of the patient's life often necessitates the empirical use of multiple broad-spectrum antibiotics, progressively escalated, and eventually including steroids, immunoglobulins, and other treatments. Experienced experts may also apply thymosin. These practices underscore the challenges of treating sepsis and the necessity of TCM intervention to support the righteous *Qi*. Therefore, Shenfu Decoction has a clinically rational basis for its application in sepsis treatment.

Shenfu Decoction, first mentioned in "*Ji Sheng Fang*" (Formulas for Succouring the Sick,《济生方》in Chinese), is composed of red ginseng and processed aconite root. We have chosen to use sun-dried ginseng in place of red ginseng to enhance its effect in greatly replenishing the primal *Qi*[12,13]; the ratio of sun-dried ginseng to aconite has been adjusted to 3:1, thereby increasing the dosage of sun-dried ginseng to strengthen the decoction's ability to benefit *Qi* and support the righteous *Qi*.[14] Sun-dried ginseng, slightly cool in nature and sweet in flavor, enters the Lung and Spleen Meridians and has the effects of greatly replenishing the primordial *Qi*, nourishing the spleen and the lung, generating fluids to quench thirst, calming the spirit, and enhancing intelligence.[15] Compared to red ginseng, sun-dried ginseng is more effective in nourishing *Yin* and generating fluids, reducing dryness, and increasing its effect in astringing and nourishing *Yin*, thus achieving a balance of nourishing both *Yin* and *Yang*. Modern pharmacological studies have found that ginseng has an immunomodulatory effect, with research indicating that its main active components, ginsenosides, can restore immune function in sepsis caused by trauma by inhibiting the activation of $CD^{4+}CD^{25+}$ regulatory T cells,[16] suggesting that using sun-dried ginseng to generate fluids and quench thirst might avoid exacerbating heat pathogens associated with excessive *Yang* warming. Moreover, its fluid-generating action is beneficial for eliminating the heat of sepsis and can counteract the heat from aconite. Aconite, extremely hot in nature and spicy-sweet in flavor, enters the heart, spleen, and kidney meridians, with effects of restoring depleted *Yang* and warming fire to assist *Yang*.[17] The active component of aconite, aconitine, has anti-inflammatory and analgesic properties, while aconite polysaccharides can promote the activation of immune responses.[18,19] When used together, they not only counteract the toxicity of aconite but also benefit *Qi*, generate fluids, restore *Yang* depletion, and secure collapse. As stated in "*Shan Bu Ming Yi Fang Lun*" (Revised and Supplemented Famous Physicians' Prescriptions and Discussions,《删补名医方论》in Chinese), "For replenishing the postnatal *Qi*, nothing compares to ginseng; for replenishing the prenatal *Qi*, nothing compares to aconite root. This is the basis of Shenfu Decoction."

Neutrophils are an important part of the body's nonspecific immune system, primarily found in the bone marrow and peripheral blood. When an infection occurs, neutrophils in the peripheral blood rapidly migrate to the site of infection, eliminating pathogens through various means such as phagocytosis or the formation of neutrophil extracellular traps.[20] However, during sepsis, the chemotactic function of neutrophils is impaired, leading to a reduced number of neutrophils reaching the site of infection, resulting in poor infection control.[21] This study shows that, compared to the model group, the number of peritoneal neutrophils in mice treated with Shenfu Decoction significantly increased, with a more pronounced effect at higher doses, indicating that Shenfu Decoction improved the chemotactic function of neutrophils toward inflammation sites in a dose-dependent manner. Compared to the model group, the low-dose group of Shenfu Decoction showed an increase in the number of blood neutrophils in mice, but the difference was not statistically significant, indicating that the mobilization of neutrophils from the bone marrow to the peritoneal cavity did not significantly impact the number of neutrophils. The number of neutrophils in the medium and high-dose groups was statistically significant compared to the model group, demonstrating the stimulatory effect of medium and high doses of Shenfu Decoction on the overall mobilization of neutrophils.

In fact, the chemotactic distance of neutrophils represents the cell's motility. TCM theory perceives it as a driving force, which is the action of *Yang* and *Qi*. Chemokines such as IL-8 and fMLP both have chemotactic effects on neutrophils, and thus, we selected these two chemotactic agents for our study. The agarose chemotaxis model, used to evaluate the chemotactic function of neutrophils, is a commonly used experimental model in recent years for research on neutrophil chemotaxis. Its maximum distance can reach up to 2,700 μm, and its direction is unrestricted, allowing for dynamic observation of cell chemotaxis directly under a microscope without the need for staining or labeling. Using the final chemotactic molecule fMLP and the intermediate chemotactic molecule IL-8 (both at optimal chemotactic concentrations) as chemotactic agents, the chemotactic distances and the differences between groups were consistent. Therefore, we believe that the direct effect of sepsis endotoxins on the chemotaxis of neutrophils and the promotive action of medium and high doses of Shenfu Decoction are consistent.

The P2X1 receptor is an ATP-gated cation channel receptor that is immediately activated upon binding with ATP, rapidly inducing the influx of cations into the cell.[22] The P2X1 receptor has the highest permeability to calcium ions, and recent studies have confirmed the expression of P2X1 receptors in peripheral blood neutrophils, eosinophils, lymphocytes, and platelets, playing a role in the immune response to pathogens.[23] Research has shown that activation of the P2X1 receptor enhances the random movement of neutrophils, but this movement lacks directionality and impairs their ability to move toward chemotactic agents, weakening the neutrophils' chemotactic ability toward infection sites. This study demonstrates that compared to the sham operation group, the expression level of P2X1 protein in the Shenfu Decoction group was significantly downregulated. Combined with the results of the

calcein staining, compared to the sham operation group, the concentration of calcium ions in neutrophils in the Shenfu Decoction group was significantly reduced. Both results are consistent, indicating that Shenfu Decoction downregulates the expression level of the P2X1 receptor in neutrophils, inhibits the influx of calcium ions, and is dose-related to Shenfu Decoction.

The P2 receptor family includes the ionotropic P2X (P2X1 to P2X7) receptors and the metabotropic P2Y (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11 to P2Y14) receptors. The P2X7 receptor has been widely reported to be activated by ATP, activating the NLRP3 inflammasome. The activated inflammasome processes the precursor of IL-1β through caspase-1, producing mature IL-1β and releasing it to cause inflammation.[24] Previous research has shown that ATP can bind to the P2Y2 receptor to regulate the chemotaxis of granulocytes and macrophages.[25] The action of Shenfu Decoction in benefiting *Qi* and warming *Yang* is analogous to the energetic and dynamic action of ATP. FPR1 encodes a G protein-coupled receptor for phagocytic cells, which mediates the chemotaxis and response of phagocytic leukocytes to bacterial formylated chemotactic peptides under inflammatory conditions.

Conclusions

In summary, sepsis is characterized by the suppression of the body's cellular immunity and chemotaxis by endotoxins, thereby exacerbating the inflammatory response. Shenfu Decoction, by tonifying *Qi* and warming *Yang*, promoting warming and transformation, can improve the chemotactic function of neutrophils. Its mechanism is unrelated to the expression levels of chemotactic factor receptors but is likely through the downregulation of P2X1 protein expression, inhibiting the influx of calcium ions. Shenfu Decoction enhances the chemotactic function of neutrophils by promoting warming *Yang*, while also nourishing *Yin* and generating fluids to regulate immune function. This makes the directionality of chemotaxis more stable and facilitates the generation of neutrophils, which is of significant importance for the treatment of sepsis and warrants further research and discussion.

Existing studies have mainly focused on the effects of ginseng and auxiliary soups on the immunomodulatory effects and inflammatory factors in septic mice. For example, ginseng and auxiliary injections can regulate immune factors and inflammatory mediators in septic mice, exerting a protective effect on septic organs. However, research into its specific mechanism of action is not yet sufficiently deep, particularly at the molecular level. The small number of experimental mice in this study limits the generalizability and credibility of the findings, making the prospect of applying ginseng and sorrel soup in the field of sepsis treatment somewhat limited.

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Conflict of interest

QQL has served as Co-editor-in-Chief, and XLX has been an editorial board member of *Future Integrative Medicine* since November 2021. The authors declare that they have no other competing interests.

Author contributions

Study concept and design (JZ, YJ), acquisition of data (WKL, WL), analysis and interpretation of data (CXW, KLW WL, JZ), drafting of the manuscript (JZ, RZ), critical revision of the manuscript for important intellectual content (JZ, XLX, RZ), administrative, technical, or material support (QQL), and study supervision (QQL, XCL). All authors have made a significant contribution to this study and have approved the final manuscript.

Ethical statement

This experiment was approved and recorded by the Animal Ethics Committee of Wuhan Traditional Chinese Medicine Hospital.

Data sharing statement

The data that support the findings of this study are available on request from the corresponding author.

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