ORIGINAL ARTICLE

Effects of Chinese Medicine Shen-Fu Injection (参附注射液) on the Expression of Inflammatory Cytokines and Complements during Post-Resuscitation Immune Dysfunction in A Porcine Model*

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ABSTRACT **Objective**: To investigate the action of Shen-Fu Injection (参附注射液, SFI) in regulating the expression of the serum complements and inflammatory cytokines synthesized and released in response to the stress of global ischemia accompanying cardiac arrest (CA) and resuscitation. **Methods**: Thirty pigs were randomly divided into the sham (n=6) and 3 returns of spontaneous circulation (ROSC) groups (n=24). After 8-min untreated ventricular fibrillation and 2-min basic life support, 24 pigs of the ROSC groups were randomized into three groups (n=8 per group), which received central venous injection of SFI (SFI group), epinephrine (EP group), or saline (SA group). Hemodynamic status and blood samples were obtained at 0, 0.5, 1, 2, 4, 6, 12, and 24 h after ROSC. **Results**: Serum concentrations of specific activation markers of the complement system C3, C4 and C5b-9 were increased during cardiopulmonary resuscitation throught 24 h after ROSC. There were intense changes of various pro-inflammatory cytokines and anti-inflammatory cytokines as early as 0.5 h after CA. Compared with the EP and SA groups, SFI treatment reduced the proinflammatory cytokines levels of interleukin (IL)-6, IL-8 and tumor necrosis factor α (TNF-α, P<0.05), and increased the anti-inflammatory cytokine levels of IL-4 and IL-10 (P<0.05). Further, SFI treatment decreased the values of C3, C4 and C5b-9 compared with the EP and SA groups. **Conclusions**: SFI, derived from the ancient Chinese medicine, has significant effects in attenuating post-resuscitation immune dysfunction by modulating the expression of complements and cytokines levels. The current study provided an experimental basis for the clinical application of a potential pharmacologic target for post resuscitation immune dysfunction.

KEYWORDS cardiac arrest, complement activation, cytokines, immune dysfunction

Survival rates following in-hospital and out-of-hospital cardiac arrest (CA) remain disappointingly low.[1] CA results in whole-body ischemia reperfusion and represents the most severe shock state, during which the delivery of oxygen and metabolic substrates is abruptly halted. The post-resuscitation phase after CA is characterized by a systemic inflammatory response similar to that observed in other systemic inflammatory conditions such as severe sepsis. It shares many features with severe sepsis, including plasma cytokine elevation with dysregulated cytokine production, the presence of endotoxin in plasma, and adrenal dysfunction, which increases the risk of multiple organ dysfunction and infection.[2] The complement system, as an important component of innate immunity, plays a pivotal role in the regulation of inflammation, host defense and antigen-specific immune response.[3] Increasing evidences demonstrate that activation of complement plays a critical role in schemia and reperfusion leading to increased vascular permeability, activation of the coagulation cascade, free-radical production, and direct tissue damage.[4,5] Bottiger, et al[6] had demonstrated significant systemic up-regulation of complement components C3a and C5b-9 during cardiopulmonary resuscitation (CPR) and early spontaneous circulation (ROSC). Cytokines, such as interleukin-8 (IL-8) and tumor necrosis factor α (TNF-α) play important roles in the pathophysiology of re-perfusion injury in patients resuscitated after CA as well as in ordinarily septic patients. Small clinical studies also have demonstrated elevated cytokine concentrations in patients resuscitated from CA and thus affords potential targets for new treatments.[7,8]

Shen-Fu Injection (参附注射液, SFI) is originated

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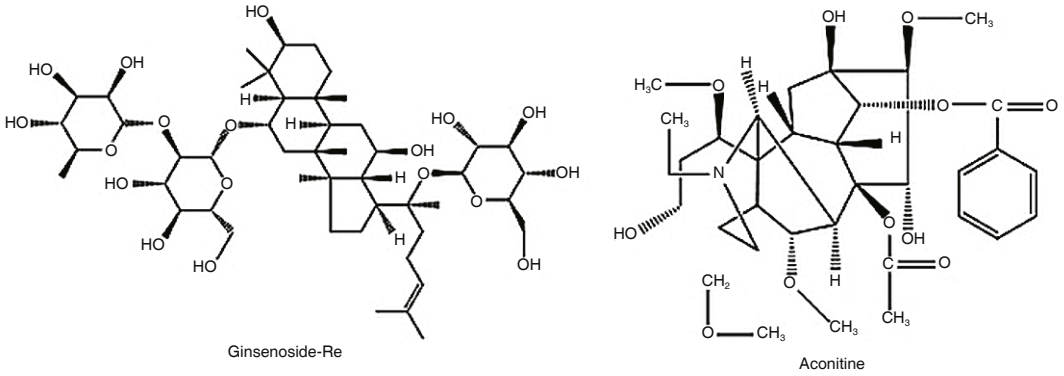

Figure 1. Chemical Structures of Ginsenoside-Re and Aconitine

from the ancient prescriptions Shenfu Formula (参附方), which is composed of *Panax ginseng* C. A. Mey. and *Radix Aconitum Carmichaeli* (Ya'an Sanjiu Pharmaceutical Co., Ltd., China). Its main active components include ginsenoside and aconite total alkaloids. Its quality is controlled strictly according to the standard of China Ministry of Public Health, and fingerprint technology was adopted in the process of production to ensure its quality.[9] Their chemical structures are shown in Figure 1. In a previous study, SFI could reduce the expression of TNF-α to block the vicious circle of inflammatory response, improve systemic microcirculation and prolong the hypoxia tolerance duration.[10] Our previous work showed that SFI was superior to dopamine in improving cardiac output, tissue perfusion, and oxygen metabolism, as well as in decreasing systemic vascular resistance, pulmonary arterial pressure, and pulmonary arterial wedge pressure in a canine shock model.[11,12]

Here we hypothesized that inflammatory cytokines and complements, synthesized and released in response to the stress of global ischemia accompanying CA and resuscitation, is one of the mechanisms involved in the post-resuscitation disturbance of immunological function. We used a porcine model of CA and CPR reflecting a realistic simulated clinical setting to examin the effects of SFI on the expressions of complements and inflammatory cytokines.

METHODS

Ethics Statement

The study experimental protocol was approved by the Committee on the Ethics of Animal Experiments of Capital Medical University (Permit No. 2010-D-013). All animals were maintained in a specific pathogen-free environment in our facility, and were fed with standard chow and had free access to water. All animal experiments were performed in a humane manner, and also in accordance with the Institutional Animal Care Instructions.

Drugs Preparation

SFI was provided by Ya'an Sanjiu Pharmaceutical Co., Ltd. (No. 110804, Ya'an, China), which contains 1 mg/mL *Panax ginseng* C. A. Mey. and 2 mg/mL *Radix Aconitum Carmichaeli*. SFI was prepared as working procedure[13]: The *Panax ginseng* C. A. Mey was crushed, extracted with ethanol 4 times under reflux (2 h/time), after soak in ethanol for 2 h, then combined the ethanol solution concentrated in a rotator evaporator in vacuo to give extract. Twenty-two compounds including 12 kinds major ginsenosides and 10 kinds rare ginsenosides were separated from the extracts of *Panax ginseng* C. A. Mey. and identified as ginsenoside Rg1, Re, Rf, Rg2, Rb1, Rc, Rb2, Rb3, Rd, F1, F3 and F5, Rk1, Rg5, Rk3, Rh4, Rg6, F4, Rg3, 20(R)-Rg3, Rh1 and 20(R)-Rh1. Different production batches of 20 kinds ginseng saponins content were range from 85–119 (Rg1), 58–89 (Re), 28–31 (Rf), 13–15 (Rg2), 198–227 (Rb1), 97–114 (Rc), 50–64 (Rb2), 8–14 (Rb3), 3–6 (F1), 36–49 (Rd), 27–42 (Rh1), 14–25 (20 (R)-Rh1), 3–6 (Rg6), 4–8 (F4), 8–11 (Rk3), 6–11 (Rh4), 21–36 (Rg3), 16–30 (20 (R)-Rg3), 8–15 (Rk1), 12–20 (Rg5) ug/mL. Aconite was extracted with water twice (2 h/time), after soak in 1% HCl water solution for 2 h. The combined solution was concentrated in vacuo, after it cooled down, added appropriate amount of ethanol, and then concentrated in a rotator evaporator to generate aconite extract.

Animal Preparation

Thirty inbred male Wuzhishan miniature pigs

aged 11 to 13 months with an average weight of 26±3 kg were used in this study (Chinese Academy of Agricultural Sciences, animal license No. Beijing 2008-050109). Our choice for the Wuzhishan mini pig is due to their characteristics similar to human beings in the histologic structures and physiology, and especially due to the highest inbreeding coefficient (more than 0.965), stable heredity and little variability between individual animals after 20 generations of inbreeding.[14] Pigs were randomly sampled by classification and divided into 4 groups, resuscitation groups: SFI, epinephrine (EP), saline (SA), *n*=8 for each group; and sham operation (*n*=6). Animals were fasted overnight but were allowed free access to water. After premedication with 0.5 mg/kg intramuscular midazolam, anesthesia was induced by ear vein injection of propofol (1.0 mg/kg) and maintained in a surgical plane of anesthesia with intravenous infusion of pentobarbital [8 mg/(kg•h)].

A cuffed 6.5-mm end tracheal tube was advanced into the trachea. Animals were mechanically ventilated with a volume-controlled ventilator (Servo 900C; Siemens, Munich, Germany) with a tidal volume of 15 mL/kg and FiO_2 of 0.21 using a tidal volume of 15 mL/kg and a respiratory frequency of 12 breaths/min with room air. End-tidal PCO_2 was measured by an inline infrared cacographic (CO_2SMO plus monitor; Respirometric Inc, Murrysville, PA). Respiratory frequency was adjusted to maintain end-tidal PCO_2 between 35 and 40 mm Hg before inducing CA. Room temperature was adjusted to 26 ℃, and body temperature was maintained at 37 ℃ under an infrared lamp. Aortic pressure was measured using a fluid-filled catheter that was advanced from the left femoral artery into the thoracic aorta. A Swan-Ganz catheter (7-Fr, Edwards Life Sciences, USA) was used to measure right arterial pressure and was advanced from the left femoral vein and flow-directed into the pulmonary artery. Continuous cardiac output was measured with a cardiac output monitor (Vigilance Ⅱ, Edwards Life Sciences). To induce ventricular fibrillation (VF), a 5-Fr pacing catheter was advanced from the right internal jugular vein into the right ventricle.

Experimental Protocol

After instrumentation, 30 min were allowed for hemodynamic stabilization. The temporary pacemaker conductor was inserted into the right ventricle through the right sheathing canal and connected to an electrical stimulator (GY-600A; Kaifeng Huanan Equipment Co. Ltd., China) programmed in the S1S2 mode (300/200 ms), 40 V, 8:1 proportion, and 10-ms step length to provide a continuous electrical stimulus until VF. VF was defined as an electrocardiogram showing waveforms corresponding to VF and a rapid decline in mean circulatory pressure toward zero. Ventilation was stopped while inducing VF, and ventilation was withheld for the entire 8-min duration of VF. After successful induction of VF, mechanical ventilation was discontinued. After 8-min untreated VF, mechanical ventilation and manual chest compressions were immediately initiated at a rate of 100 compressions per minute with mechanical ventilation at an FiO_2 of 100%.

CPR was performed by the same CPR technician from our laboratory, who compressed the porcine chest approximately one third of anteroposterior diameter. The quality of chest compressions was controlled by a Heart-start MRx Monitor/Defibrillation with Q-CPR (Philips Medical Systems, Best, Holland).[15] Eight minutes of VF was chosen because it is clinically relevant relative to emergency response system arrival and because by logistic regression models, this time period offers a realistic chance of influencing survival.[16] Ventilation was delivered by a bag respirator with room air, and the compression-to-ventilation ratio was 30:2. Furthermore, the investigators involved in data recording, data entry, and data analysis were also blinded to the allocation. After 2-min CPR, pigs were randomized divided into 3 groups, then receive, respectively, central venous injection of SFI (1.0 mL/kg), EP (0.02 mg/kg) and SA.

The study was blinded as to the medication used, and only the principal investigator, who did not take part in any resuscitation effort, knew the assignment of each animal. If VF persisted after 10 cycles of CPR (about 4 min), a 100 J (about 4 J/kg) shock (SMART Biphasic) was delivered. If the defibrillation attempt failed to attain ROSC, manual chest compressions were rapidly resumed for a further 2 min followed by a second defibrillation attempt. The second and subsequent shocks were delivered 150 J. Resuscitation was continued until ROSC was achieved, or for longer than 30 min if ROSC was not achieved. The same procedure without CA initiation was achieved in the sham group, including induction of anaesthesia, electrode positioning, mechanical respiration, 8-min ventilation withheld, and monitoring of physiological parameters.

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ROSC was defined as consecutive 10 min of maintenance of systolic blood pressure at 50 mm Hg. If spontaneous circulation was not restored within 30 min, we regarded the animal as dead.[17] All the animals received normal saline (10 mL/kg·h) intraoperatively to replenish fluid losses. After successful resuscitation, the animals were mechanically ventilated with 100% oxygen. With the exception of one jugular vein sheath that was used for fluid administration, all other vascular sheaths and end tracheal tubes were removed after a 6-h intensive care period. The animals were allowed to recover from anesthesia, and were then placed in observation cages and monitored for a further 18 h. The animals were euthanatized with 10 mL of 10 mol/L potassium chloride intravenously following a bolus of 100 mg of propofol intravenously.

Hemodynamic Measurement

Hemodynamic parameters, including cardiac output (CO) and mean arterial pressure (MAP), were measured continuously, and we recorded the values at baseline and 0.5, 1, 2, 4, and 6 h after ROSC. Oxygen metabolism parameters, including oxygen delivery (DO_2) and oxygen consumption (VO_2), were calculated.

Assessment of Complement Activation and Cytokine Analyses

Blood samples were collected for laboratory testing at baseline, 30 min after ROSC (ROSC 0.5 h), 2 h after ROSC (ROSC 2 h), 4 h after ROSC (ROSC 4 h), 6 h after ROSC (ROSC 6 h), 12 h after ROSC (ROSC 12 h), and 24 h after ROSC (ROSC 24 h).

Measurements of Cytokines Concentrations

Immediately before induction of VF and at 24 h intervals following ROSC, venous blood was sampled, placed in chilled (0 ℃) heparinized sterile tubes, and centrifuged at 2,500 r/min for 10 min. Plasma was immediately separated and stored at 80 ℃ until analysis. IL-6, IL-8, TNF-α, IL-4 and IL-10 concentrations were determined by a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) using commercially available kits specific for these porcine cytokines (R&D Systems, Minneapolis, MN). The mean minimum detectable concentration of the immunoassay for TNF-α is 3.7 and 10 pg/mL for IL-8 and IL-6. The ELISA was specific for the cytokines being assayed. Cytokine concentrations in these specimens were compared with those in samples obtained after instrumentation (approximately 1.5 h) and before VF induction. Cytokine concentrations were obtained from a standard curve. Duplicate readings were obtained for all samples and the means were calculated.

Measurements of Complement Activation

Activation of the complement cascade was assessed by measuring the concentrations of the activation-specific split-product anaphylatoxin C3 (desArg) and C4 (desArg) in serum, which were measured by ELISA according to the manufacturers' instructions (Progen, Heidelberg, Germany) and the concentrations of the terminal complement complex C5b-9. C5b-9 was measured by ELISA on microtiter plates (Nunc, Wiesbaden, Germany), using as capture antibody a monoclonal immunoglobulin G recognizing a neoantigen specific for the membrane attack complex (aE11, Diatec AS, Oslo, Norway).[10] After blocking unspecific binding sites, appropriate dilutions of the samples were incubated. Detection of C5b-9 was achieved by anti-human C5 (Dako, Hamburg, Germany), followed by the appropriate peroxidase-labeled third antibody (Dianova, Hamburg, Germany).

Statistical Analysis

The experimental data were analyzed by SPSS 17.0. The results are expressed as mean ± standard deviation, and Student's *t* test was used for comparisons between two groups. Differences at different time points within groups were compared with repeated-measures ANOVA. A two-tail value of $P<0.05$ was considered significant.

RESULTS

Animal Survival

Eighteen of 24 animals were successfully resuscitated, including 5, 6 and 7 in the SA, EP and SFI groups, respectively. By comparison, the number of electric shock, defibrillation energy and time to ROSC were significantly lower in the EP and the SFI groups than in the SA group ($P<0.05$), but there was no difference between the EP and the SFI groups ($P>0.05$, Table 1).

Table 1. Resuscitation Outcome ($\bar{x} \pm s$)

Group	Number of ROSC	Number of shock	Energy of shock (J)	Time to ROSC (min)
SA	5	5.5 ± 2.5	795.0 ± 375.7	10.0 ± 3.8
EP	6	2.8 ± 1.7*	312.7 ± 134.3**	6.0 ± 2.2**
SFI	7	2.6 ± 1.0*	332.5 ± 168.4**	5.0 ± 1.7**

Notes: *$P<0.05$, **$P<0.01$, compared with the SA group

Hemodynamic and Oxygen Metabolism Status

MAP and CO did not differ significantly among the four groups at baseline (P>0.05). After successful resuscitation, the values of MAP were significantly decreased in the SA group at 30 min compared with the EP group (P<0.01), 1 and 2 h after ROSC values compared with the sham group (P<0.05). In contrast, MAP was significantly increased in the SFI group compared with the EP group at 1, 2 and 6 h after ROSC (P<0.05); however, there were no differences in MAP between the SFI and EP groups at 30 min and 4 h after ROSC (Figure 2A). The values of CO were significantly decreased in the SA (P<0.01), EP and SFI groups after ROSC compared with the sham group (P<0.05). CO was significantly higher in the SFI and EP groups than the SA group at 4 and 6 h after ROSC (P<0.05). However, there were significant differences in CO between the SFI and the EP groups at 6 h after ROSC (Figure 2B). DO_2 and VO_2 did not differ significantly among the four groups at the baseline (P>0.05). DO_2 and VO_2 after ROSC declined significantly in resuscitation groups at 30 min (P<0.05) and at 2, 4 and 6 h after ROSC (P<0.01). However, DO_2 was significantly higher in the SFI group than the EP group at 4 and 6 h after ROSC (Figure 2C); VO_2 was significantly higher in the SFI group than the EP group at 2 and 4 h time points after ROSC (Figure 2D).

Serum Cytokines Levels at Baseline and throughout 24 h after ROSC

Baseline of serum cytokines measurement did not differ significantly among the four groups (P>0.05; Figures 3 and 4). There were also no significant differences in cytokines among the four groups until 0.5 h after ROSC. The results showed that the proinflammatory cytokines levels of IL-6, IL-8, TNF-α and anti-inflammatory cytokine IL-4 were markedly increased in the SA, EP and SFI groups compared with the sham group throughout 24 h after ROSC, while anti-inflammatory cytokines of IL-10 was significantly decreased (P<0.05 or P<0.01). Furthermore, the levels of IL-6, IL-8 and TNF-α were significantly lower in the SFI group compared with the EP group and the SA group at 2, 4, 6 h after ROSC (P<0.05). However, there was no significant difference between the groups at any other time points (Figure 3). The values of IL-4 and IL-10 were significantly higher in the SFI group than in the SA and the EP groups at 0.5, 4 and 6 h after ROSC (P<0.05); In addition, IL-4 and IL-10 were higher in the EP group than in the SA group at 0.5, 2, 4 and 6 h (P<0.05, Figure 4).

Complement Activation

The measured concentrations of C3, C4 and C5b-9 are demonstrated in Figure 5. A marked activation of the complement system, as indicated by increased concentrations of C3, C4 and SC5b-9 were markedly increased in the SA, EP and SFI groups compared with the sham group throughout 24 h after ROSC (P<0.05 or P<0.01). The value of C3 was significantly decreased in the SFI group and the EP group compared to and SA

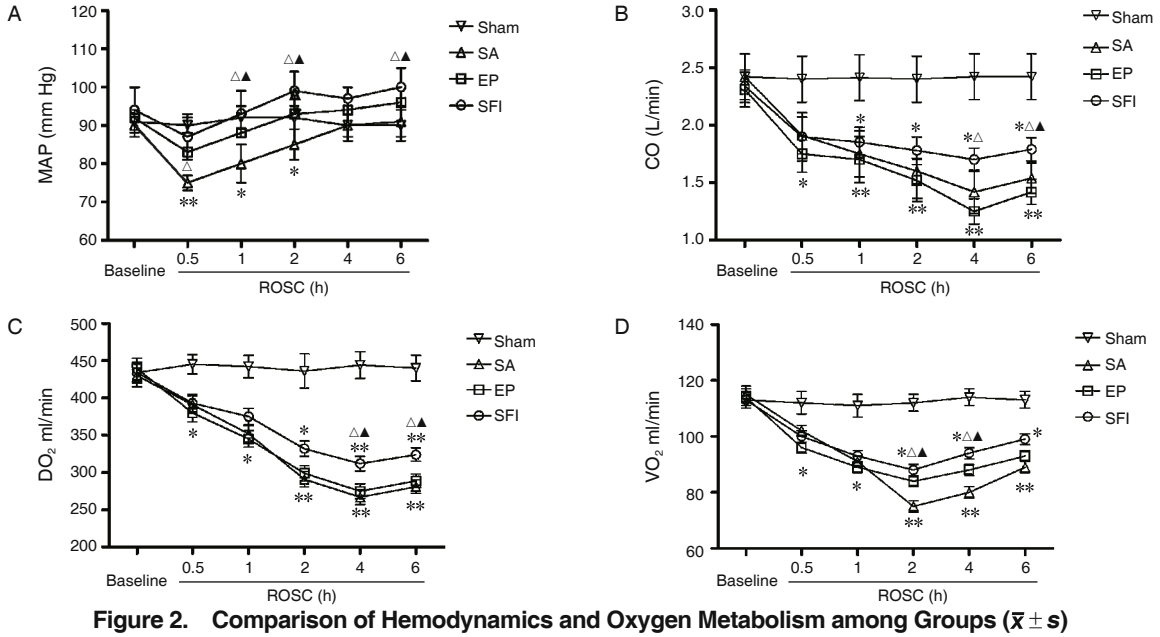

Figure 2. Comparison of Hemodynamics and Oxygen Metabolism among Groups ($\bar{x} \pm s$)

Notes: A: mean aortic pressure (MAP); B: Cardiac output (CO); C: Oxygen delivery (DO_2); D: Oxygen consumption (VO_2). $^{*}P$<0.05. $^{**}P$<0.01, compared with the sham; $^{\triangle}P$<0.05, compared with the SA group; $^{\blacktriangle}P$<0.05, compared with the EP group; the same below

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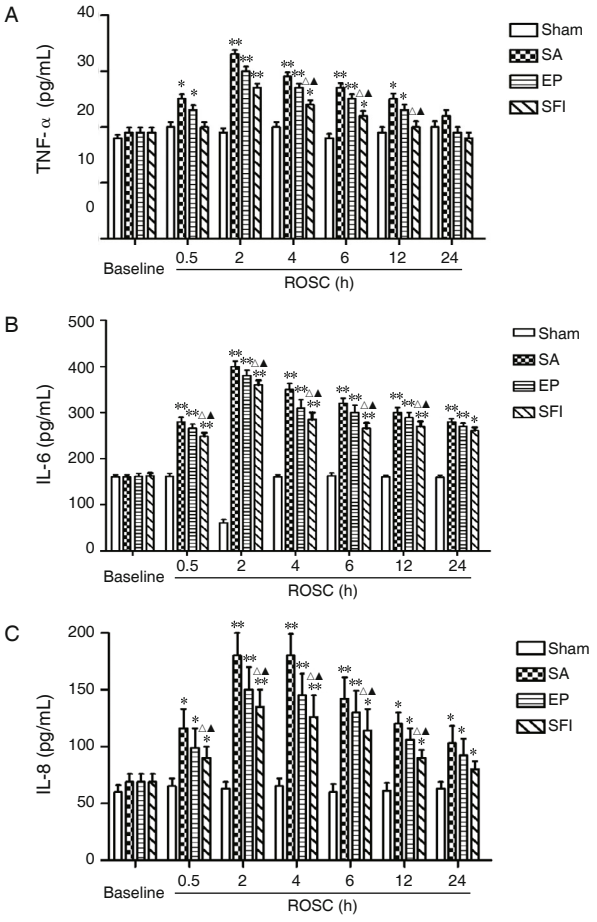

Figure 3. Comparison of Pro-Inflammatory Cytokines IL-6, IL-8, and TNF-α by ELISA ($\bar{x} \pm s$)

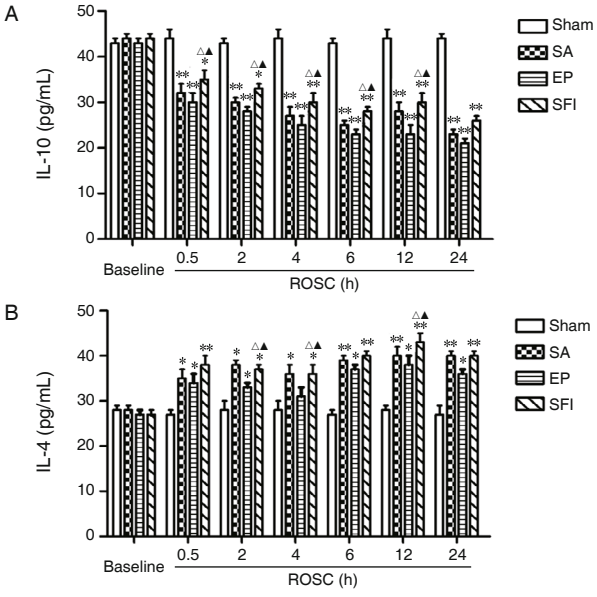

Figure 4. Comparison of Anti-Inflammatory Cytokines IL-4 and IL-10 by ELISA ($\bar{x} \pm s$)

group at 2, 4, and 6 h after ROSC (P<0.05). However, there were no differences in C3 between the SFI and EP groups at 30 min and 2 h after ROSC (Figure 5). The concentration of C4 was significantly lower in the SFI group than in the EP group at 2, 4, and 6 h after ROSC (P<0.05, Figure 5). The concentration of C5b-9 were significantly lower in the SFI group than the EP group at all time points except 30 min and 24 h time points after ROSC (P<0.05, Figure 5).

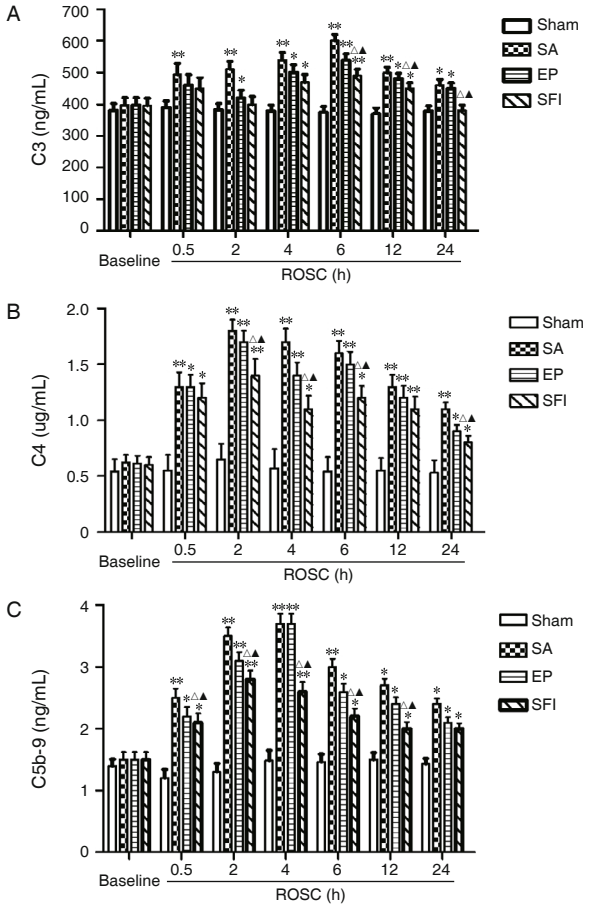

Figure 5. Comparison of C3, C4 and C5b-9 Concentrations among Groups ($\bar{x} \pm s$)

DISCUSSION

Ischemia re-perfusion during CA and CPR has been known to cause oxidative stress and contribute to post-cardiac arrest syndrome.[18] Some researches indicate that post-resuscitation disease related to an early systemic inflammatory response as observed in severe sepsis.[2] Furthermore, cardiovascular function and hemodynamic disorders frequently occurred after ROSC, which can lead to shock and immunosuppression caused by changes in the normal levels of inflammatory cytokines.[18] In the present study, pigs that underwent CA all presented with a marked activation of complement and an increased concentration of proinflammatory cytokines during early reperfusion after CA. These changes can be important factors in the etiology of

reperfusion tissue injury and microcirculatory reperfusion disorders after CA. During CPR, a compensatory increase in systemic oxygen extraction occurs, which leads to significantly decreased central or mixed venous oxygen saturation.[19] Inadequate tissue oxygen delivery can persist even after ROSC because of myocardial dysfunction, hemodynamic instability and microcirculatory dysfunction. Furthermore, accumulated oxygen debt leads to activation of immunologic pathways and systemic inflammation.[20] Our results showed that SFI group had a better outcome in hemodynamic and oxygen metabolism parameters compared to the EP and SA groups, suggesting that SFI can protect myocardial tissue from injury and improve postresuscitation myocardial dysfunction.

In an actual study, post-resuscitation disease in patients with successfully resuscitated after CA was associated with a systemic inflammatory response similar to the immunologic profile observed in patients with severe sepsis.[2] Components of the systemic inflammatory response syndrome include ischemia and reperfusion syndrome. During the ischemic phase, ischemia-induced hypoxia is thought to be a key cause of tissue and cell damage. Under these conditions oxygen debt precludes oxidative phosphorylation in the mitochondria, leaving the anaerobic pathway as the only available source of ATP, causing the cellular ATP levels to drop. This systemic post-resuscitation response constitutes a sepsis-like syndrome in which systemic inflammation and immune paralysis (endogenous immunosuppression) may reinforce each other, leading to an increased risk of subsequent nosocomial infections and fatal multiorgan failure.[21,22] Based on these possibilities, we measured proinflammatory cytokines (IL-6, IL-8, TNF-α) and anti-inflammatory cytokines (IL-4 and IL-10) levels in ROSC animals after CA in the present study. Serum levels of IL-6, IL-8 and TNF-α were confirmed to increase after ROSC, peaking within 2 h and within 4 h, respectively. Increases of serum cytokines by the neutrophils activated by such stresses as infection and lipopolysaccharide injection have been reported previously,[22,23] in which the serum IL-8 level increased after the stresses and peaked within 2–3 h and the serum TNF-α level increased after the stress or infection, peaking within 4 h. Anti-inflammatory cytokine of IL-4 were markedly increased in the SA, EP and SFI groups compared with the sham group (P<0.05 or P<0.01) throughout 24 h after ROSC, while IL-10 was significantly decreased.

Complement activation generates the pro-inflammatory peptides anaphyla-toxins C3a and C5a and leads to cell injury by insertion of the cytolytic membrane attack complex C5b-9.[24] Activation of complement, and related tissue damage and microcirculatory reperfusion disorders, has been demonstrated in various experimental models of ischemia and reperfusion.[25] Clinical and experimental studies in several organ systems have shown that re-perfusion following ischemia results in local activation of the complement system.[26] Cell membranes, including those present on endothelial cells, contain proteins such as decay-accelerating factor and membrane cofactor protein, which protect against complement attack. Damage to these protective proteins is one of the possible mechanisms by which complement activation is allowed to proceed during ischemia and reperfusion.[27] Complement activation after CA is potentially beneficial, aiding to the host defense against infection and facilitating wound healing, provided it is well balanced and controlled. However, excessive complement activation contributes to organ dysfunction and increased morbidity and mortality in the post-resuscitation period. After CA, three pathways can activate the complement system. Direct contact between heparinized blood and the synthetic surfaces of the extracorporeal perfusion circuit activates proteins of the classical complement pathway.[28] Generation of C3b by the classical pathway activates the alternative pathway. Although the classical pathway proceeds in sequential steps, the alternative pathway creates a positive feedback loop that amplifies cleavage of C3 by membrane-bound C3 convertase to membrane-bound C3b and C3a. Complement activation occurs early in cases of IRI injury leading to the release of biologically active substances including the complement factor 3a (C3a) and 5a (C5a), and the cytolytic terminal membrane attack complement complex C5b-9 (TCC).[29] Activation of the complement cascade results in increased concentrations of C3a and C5a and the generation of C5b-9.

Our group is interested in identifying and testing in our porcine model of CA and resuscitation new valid interventions to mitigate ischemia and re-perfusion injury following reperfusion. In some previous studies, the immunomodulatory effects of SFI could reduce the expression of TNF-α to block the vicious circle of inflammatory response, improve systemic microcirculation, prolong the hypoxia tolerance duration, increase blood supply and oxygenation, and promote

the release and application of oxygen, inhibit xanthine oxidase directly, protect the SOD activity, and resist lipid peroxidation.[9,10] Therefore, we further analyzed the effects of SFI on the post-resuscitation immune function on modulating the expression of serum cytokines and complement system. "Shenfu Decoction" has been commonly used in China for nearly 800 years. SFI is a typical form of Shenfu Decoction for intravenous medication, whose main components include ginsenoside and aconitine.[30] A previous study had demonstrated that ginsenoside could stimulate immune function, improve both specific and nonspecific immunity, and influence both humoral immunity and cell immunity. They showed that total ginseng soap could promote the biosynthesis of proteins.[31] *Aconitum carmichaeli* Debx. could also improve immune function by enhancing the adrenocortical system.[32]

Furthermore, previous studies undertaken in our laboratory showed that the effects of SFI were based on aconitine properties, supplemented by ginsenoside, which can scavenge free radicals, improve energy metabolism,[33] inhibit inflammatory mediators, suppress cell apoptosis, and alleviate mitochondrial damage.[11] In the present study, we revealed that the levels of IL-6, IL-8 and TNF-α were significantly decreased in the SFI group than in the SA and EP groups at 2, 4, 6 h after ROSC. At the same time, the values of IL-4 and IL-10 were significantly higher in the SFI group than in the SA and EP groups at 0.5, 4 and 6 h, which demonstrated that SFI can attenuate post-resuscitation immune dysfunction through modulating the expression of serum cytokines. Further, in our study, SFI treatment decreased the values of C3, C4 and C5b-9 compared with the EP and SA groups, also indicating that a critical role of the SFI in post-resuscitation immune dysfunction. It is of interest to investigate the detailed mechanisms by which SFI regulating the immune dysfunction in the future.

Our data demonstrated a marked activation of complements and different changes of cytokine concentrations during cardiopulmonary resuscitation after CA. Furthermore, our results showed that SFI could antagonize TNF-α, IL-6 and IL-8 release, increase IL-4 and IL-10 expression on early systemic inflammatory response after CA, promoting recovery of immune function with two-way adjustment capability. It would be interesting to determine in the clinical setting whether the new therapeutic approaches based on the findings in this study would improve outcome after CA.

In the interpretation of our findings, repetitive electrical shocks themselves may increase the severity of post-resuscitation myocardial dysfunction in settings of myocardial ischemia.[34] Because our studies were performed in an animal model in the absence of underlying cardiovascular disease, direct applicability to human patients cannot be ensured. Optimal doses and methods of administration of SFI also deserve additional investigation. This information would be useful in applying the use of SFI to CPR.

Conflict of Interest

The authors have declared that no actual or potential conflict of interest in relation to this article.

Author Contributions

Li CS conceived and designed the experiments; Zhang Q, Li CS, Wang S, and Gu W performed the experiments; Zhang Q analyzed the data; Zhang Q and Wang S contributed to reagents, materials, and analysis tools; Zhang Q and Li CS wrote the paper.

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