Basic Investigations

Study on Modified Shengmai Yin Injection for Prevention and Treatment of Brain Impairment in Endotoxin Shock Rats

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Objective: To examine the effects of modified Shengmai Yin on invigorating vital energy, promoting blood flow, and protection against neural impairment in an endotoxin-induced shock rat model.

Methods: Ninety-six SD rats were randomly divided into four groups: sham operation (saline 20 ml/kg), shock model (lipopolysaccharide, LPS, 8 mg/kg), Reformed Shengmai Yin (加味生脉饮 Pulse-activating Decoction) (LPS 8 mg/kg + reformed Shengmai Yin Injection 10 ml/kg), and dexamethasone (LPS 8 mg/kg + dexamethasone 5 mg/kg) groups. Each group was subdivided into 1 h, 2 h, 3 h, and 6 h time points for observation. The carotid artery was separated and connected with a biological functional system to monitor mean arterial pressure (MAP). Brain water levels, malonaldehyde (MDA) content, and superoxide dismutase (SOD) activity were also determined.

Results: In the shock model group, MAP was progressively decreased after injection of LPS, brain water and MDA contents were increased, brain SOD activity was decreased, and capillary vessel edema in brain tissue was also observed. All these parameters were improved significantly in both treatment groups, although the effects were more marked with Shengmai Yin than with dexamethasone.

Conclusion: Modified Shengmai Yin exhibits strong anti-shock and neuroprotective effects against Endotoxin-induced shock.

Keywords: endotoxin shock; brain impairment; invigorate vital energy, promote blood flow, and detoxify; free radicals; Shengmai Yin (Pulse-activating Decoction)

Septic shock is a commonly-encountered critical clinical illness. Bacterial endotoxin and excessive inflammatory mediators released by mononuclear macrophages from endotoxin-stimulation are the main pathogenetic mechanisms of shock. 1 Recuperating depleted yang to rescue patients from collapse is suitable for prevention and treatment of shock in the early phase. To counter the complicated pathologic state of shock in the middle and late phases, an injection of Reformed Shengmai Yin (加味生脉散注射液 Jia Wei Sheng Mai Zhu She Ye) with an effect of invigorating vital energy, promotion of blood flow, and detoxification has been developed, which is scientifically prepared with the classical recipe Shengmai Yin (生脉饮 Pulse-activating Decoction) with Tao Ren (桃仁 Semen Persicae), Hong Hua (红花 Flos Carthami), Shi Chang Pu (石菖蒲 Rhizoma Acori Graminei), and Bai Hua She She Cao (白花蛇舌草 Herba Hedyotis Diffusae) added. In the present study, the effect of injection of Reformed Shengmai Yin on mean arterial pressure (MAP) and on water and malonaldehyde (MDA) contents, superoxide dismutase (SOD) activity, and structural changes in brain tissue were examined in a rat model of endotoxin shock.

MATERIALS AND METHODS

Experimental Animals, Chemicals, and Equipment
Ninety-six healthy adult Sprague-Dawley rats (48...
females and 48 males; body weight 180–220 g) were provided by Beijing Weitong Lihua Laboratory Animals and Techniques Ltd Co. (Certificate: ZCXX (京) 2008–0003).

Chemicals: Lipopolysaccharide (LPS, E Coli O 127B8, Sigma); Reformed Shengmai Injection (prepared by Chemistry Faculty of Capital Normal University, 10 ml/ampul); Dexamethasone (DEX, Beijing Double Crane Pharmaceutical Ltd Co., 5 mg/ampul, Batch No. 311818A); Chloral hydrate (Beijing Chemical Reagents Co., Batch No. 20050528); Coomassie brilliant blue kit, MDA, and SOD kits (Nanjing Jiancheng Bioengineering Institute); Analytically pure absolute alcohol, paraformaldehyde, and xylene (Beijing Chemical Reagents Co.).

Equipment: BL-420E+ biology function experiment system (PHILIPS Co.; MP20/junior); GR-200 electron analytic balance (No:14213185; A&D Ltd. Co.); CH light microscope (Olympus Co.); GZX-DHG electric heat constant temperature drying oven (Shandong Weifang Medical Instruments Factory); EG-1150C tissue embedding machine and RM-2135 microtome (Leica Co.); 722 s spectrophotometer (Shanghai Precision Scientific Instruments Ltd. Co.).

Experimental Groups and Methods

The 96 rats were randomly divided into four groups: sham operation group, shock model group, Reformed Shengmai Yin group, and dexamethasone group. Each group was subdivided into four observation points (1 h, 2 h, 3 h, 6 h), with six rats at each time. All rats were anesthetized by injection of 10% chloral hydrate (0.35 ml/100g) into the abdominal cavity. The left common carotid artery was separated and a PE50 tube with heparin was inserted. The tube was connected with a three-limb tube to a BL-420E biology function experiment system via a YH-100 pressure energy transducer to monitor MAP. The right external jugular vein was separated and a PE50 tube with heparin that was linked to a transfusion system was inserted. When the blood pressure stabilized, it was recorded once every 10 min until 6 h when the experiment was finished. Ten minutes after successful surgery, sham operation group received normal saline (20 ml/kg), the shock model group received LPS (8 mg/kg), the dexamethasone group received LPS (8 mg/kg) + dexamethasone (5 mg/kg), and the Reformed Shengmai Yin group received LPS (8 mg/kg) + Reformed Shengmai Yin (10 ml/kg). The transfusion capacity for all groups was 20 ml/kg. Rats that exhibited a 25–30% decrease in MAP from baseline after LPS intravenous infusion were regarded as acute endotoxin shock animals.

Sampling

Rats were killed by decapitation and the brains removed. The middle cortex of the left cerebrum was isolated and homogenized with physiologic saline in an ice-bath, and centrifuged for 10 min at 4°C (3500 r/min). The supernatant was removed and 10% homogenate was prepared and kept at -70°C for biochemical determination. The right brain tissue was fixed in 10% neutral formalin for preparation of morphological specimens.

Observation Indexes

1. General state and survival rate of animals.
2. Mean arterial pressure (MAP): Blood pressure at each time point (0 h, 1 h, 2 h, 3 h, 6 h) and the waveforms of blood pressure were recorded. MAP (kPa) = diastolic pressure + 1/3 pulse pressure.
3. Water content of brain tissue: Left cortex (approximately 100 mg) was removed and weighed with an electronic balance (scale division <0.1 mg), and roasted two to three times in an electron heat constant temperature drying oven at 105°C for 24 h each time until constant weight (i.e., the difference of specimen dry weights at end of each repeat drying was less than 0.2 mg). Water content of brain tissue = (humid weight – dry weight) / humid weight × 100%.
4. SOD activity determination: A 10% homogenate of brain tissue was diluted into 1% with normal saline, and centrifuged at 4000 rpm for 10 min. SOD activity in the supernatant (20 l) was performed according to kit description, and protein level in the supernatant (50 l) was determined using the Coomassie brilliant blue method. SOD activity in each gram protein was calculated.
5. MDA content determination: 10% homogenate of
brain tissue was prepared and centrifuged at 4000 rpm for 10 min. MDA content in the supernatant (100 μl) was performed according to kit description, and protein level in 1% supernatant (50 μl) was determined using the Coomassie brilliant blue method. The content of MDA in each gram protein was calculated.

6. Pathological examination: At each observation point, the brain was quickly removed and then fixed in 4% paraformaldehyde at 4°C. After routine dehydration, clearing, paraffin imbedding, sectioning (5 μm thickness), and hematoxylin-eosin (HE) staining, pathological examination was then performed under a light microscope.

Statistical Analysis
All data were analyzed by SAS8.2 software. First, a homogeneity test for variance was conducted. If the variance was regular, analysis of variance was performed directly. If the variance was irregular, then analysis of variance was performed when variance was made regular after parameter replacement and homogeneity test for variance. Results were expressed as $\bar{X} \pm s$, and analysis of variance (ANOVA/LSD) was used for analysis of the difference of inter-groups. $P<0.05$ was considered statistically significant.

RESULTS

General State of Health
The general state of health in sham operation rats was good, with stable breath and normal temperature of limbs, without marked changes before and after the experiment. In the shock model group, animals exhibited polypnea, cyanotic lips, a fall in body temperature, cold limbs, and shivering after LPS injection. In the dexamethasone group and the Reformed Shengmai Yin group, the general state of health was improved compared with the shock model group, and the polypnea was obviously relieved. However, animals still exhibited low spirit, diarrhea, and piloerection.

Dynamic Changes in MAP
There were no marked changes in MAP in the sham operated animals during the experimental process. In the shock model group, MAP fell quickly after LPS injection, slightly increased within 30 min, and then gradually declined until death after 90–120 min. In the treatment groups the pattern of blood pressure change was similar to that in the shock model group, although the degree of hypotension was significantly reduced significantly ($P<0.05$). There were no differences in MAP between the dexamethasone group and the Reformed Shengmai Yin group ($P>0.05$, Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>0 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
<th>360 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>119.4±3.2</td>
<td>113.2±3.1</td>
<td>109.5±2.1</td>
<td>95.6±2.3</td>
<td>87.3±1.4</td>
</tr>
<tr>
<td>Shock model</td>
<td>101.2±2.3</td>
<td>65.4±3.5</td>
<td>59.1±3.2</td>
<td>50.2±2.1</td>
<td>42.5±1.2</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>118.5±3.4</td>
<td>79.4±7.8</td>
<td>70.3±9.2</td>
<td>66.1±2.5</td>
<td>65.3±2.7</td>
</tr>
<tr>
<td>Reformed Shengmai Yin</td>
<td>120.2±4.8</td>
<td>89.8±9.6</td>
<td>84.9±4.7</td>
<td>77.4±7.8</td>
<td>68.9±7.4</td>
</tr>
</tbody>
</table>

Notes: ^$P<0.05$, compared with the sham operation group; *$P<0.05$, compared with the shock model group.

Mortality
The mortality in the shock model group at 6 h was 83.3%, which was significantly higher than that in the dexamethasone group and the Reformed Shengmai Yin group ($P<0.05$). There was no difference in mortality between the dexamethasone group and the Reformed Shengmai Yin group ($P>0.05$).

Brain Tissue Moisture
At 6 h after shock the brain water content in the shock model group increased significantly compared with the sham operation group ($P<0.01$). The brain water content in both treatment groups was significantly lower than that in the shock model group ($P<0.01$). There were no differences in brain water content between the dexamethasone group and the Reformed Shengmai Yin group ($P>0.05$), indicating that both drugs could significantly relieve cerebral edema of the shock group rats (Table 2).
Table 2. Effect of Reformed Shengmai Yin on brain tissue moisture in endotoxin shock rats (X ± s, n=6, %)

<table>
<thead>
<tr>
<th>Group</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
<th>360 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>77.62±0.39</td>
<td>77.86±0.23</td>
<td>78.01±0.11</td>
<td>78.30±0.43</td>
</tr>
<tr>
<td>Shock model</td>
<td>80.99±0.34▲▲</td>
<td>81.61±0.77▲▲</td>
<td>82.07±0.32▲▲</td>
<td>82.69±0.28▲▲</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>79.61±0.41★★★★ ★★★★</td>
<td>80.34±0.11★★★★ ★★★★</td>
<td>80.78±0.19★★★★ ★★★★</td>
<td>81.23±0.54★★★★ ★★★★</td>
</tr>
<tr>
<td>Reformed Shengmai Yin</td>
<td>78.04±0.23★★★★ ★★★★</td>
<td>79.56±0.42★★★★ ★★★★</td>
<td>79.88±0.21★★★★ ★★★★</td>
<td>80.36±0.16★★★★ ★★★★</td>
</tr>
</tbody>
</table>

Notes: ★P<0.05, ★★P<0.01 compared with the sham operation group; ★★★P<0.05, ★★★★P<0.01 compared with the shock model group.

SOD Activity and MDA Content in Brain Tissue
As there were no significant changes in SOD activity or MDA content in the brain tissue between any of the recovery points, data for all time points within each group were combined. Compared with the sham operation group, SOD activity of brain tissue was significantly decreased (P<0.01) and MDA content of brain tissue significantly increased (P<0.01) in the shock model group. Compared with the shock model group, SOD activity in the Reformed Shengmai Yin group and the dexamethasone group were significantly increased (P<0.01 for both) and MDA content was significantly decreased (P<0.01, P<0.05, respectively). The increased SOD activity and decreased MDA content in the treatment groups was more pronounced in the Reformed Shengmai Yin group (P<0.05; Table 3).

Table 3. Effect of Reformed Shengmai Yin on SOD activity and MDA content of brain tissue in endotoxin shock rats (X ± s, n=24)

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (nmol/mg prot)</th>
<th>SOD (U/mg prot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>4.13±0.04</td>
<td>70.43±1.79</td>
</tr>
<tr>
<td>Shock model</td>
<td>9.02±1.19▲</td>
<td>37.15±2.92★</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>6.96±1.08★★★</td>
<td>44.15±2.22★★★</td>
</tr>
<tr>
<td>Reformed Shengmai Yin</td>
<td>5.67±0.89★★★★</td>
<td>50.82±4.76★★★★</td>
</tr>
</tbody>
</table>

Notes: ★P<0.05, ★★P<0.01 compared with the sham operation group; ★★★P<0.05, ★★★★P<0.01 compared with the shock model group.

Pathological Changes of Brain Tissue
Using H&E staining, in the sham operation group the structure of the cerebral cortex was clear and had good integrity. Furthermore, nerve cells lined up in order with abundant and hypochromatic cytoplasm, the nucleus was located in the center, and there were clear nucleoli. In the shock model group, obvious congestion of meningeal blood vessels, peripheral edema of a part of the brain white matter, widening of the gap surrounding small vessels, and punctiform nervous cell necrosis were observed at 1 h, 2 h, and 3 h recovery. By 6 h the congestion of meningeal blood vessels was most obvious, with peripheral edema of ectocineerea small vessels and many foci of nervous cell necrosis. Compared with the shock model group, pathological changes in the two treatment groups were partly reduced.

DISCUSSION
In the present study the authors compared the effects of Reformed Shengmai Yin and dexamethasone in an endotoxin shock rat model in rats. Endotoxin is a major component of the pathogenetic mechanism of septic shock, and MAP is one of the most effective hemodynamic indexes reflecting shock. After LPS injection, MAP was decreased to 20% of baseline at 5 min, followed by slight recovery at 30 min, and then progressive decline until 240 min, indicating successful duplication of the endotoxin shock model.

The ability of endotoxin is to cross the blood brain barrier remains controversial, and it remains possible that endotoxin can directly affect cerebral nerve cells following high-dose treatment or when the blood brain barrier is weakened by cerebral disease. During cerebral ischemia, hypoxia, or imbalanced ion pump function induced by septic shock, high levels of sodium and chloride ions are considered to enter brains cell from the extracellular fluid, leading to brain cell edema. Brain edema is a principal pathological change of brain damage in septic shock. Decreased blood pressure is the major pathogenic mechanism of shock, causing ischemia and hypoxia of organs. As such, monitoring MAP is one
of the most effective hemodynamic indexes for understanding the pathogenic condition of shock.²

Release of oxygen free radicals is an important factor contributing to brain impairment following endotoxin shock. Under physiological conditions, free radicals in the brain play a role in maintaining normal cerebral function. However, under pathological conditions such as cerebral trauma and cerebral ischemia, high levels of oxygen free radicals are produced in the brain. As these free radicals are not rapidly removed they can cause serious damage of the brain cellular membrane. MDA content can indirectly reflect changes of oxygen free radical content, and SOD activity can be used to evaluate the antioxidant capability of brain tissue. As well as causing direct injury to brain tissues and cells, free radicals can trigger numerous damaging signal transduction pathways including brain excitatory amino acid toxicity.³,⁴ In the present study the water and MDA content of brain tissue was significantly increased and the SOD activity significantly decreased in the shock model group. Treatment with Reform Reformed Shengmai Yin significantly reduced the decrease in water and MDA content and the increase SOD activity following endotoxic shock, and ameliorated the brain blood capillary edema. These data suggest that the protective mechanism of Reform Shengmai Yin was related to its regulation of free radicals.

Invigorating qi, promotion of blood flow, and detoxification is a new Chinese medicinal therapy used for treatment of microcirculatory failure in the late phase of shock. Recuperation of depleted yang to rescue the patient from collapse, supplementing qi and nourishing yin to induce resuscitation and treating prostration, and promoting blood flow and restoring pulse have been used in the early phase of shock, although these treatments are insufficient for the complicated pathological changes in the irreversible stage of shock.⁵ As the complete pathogenesis of shock remains unclear, and due to limited Western medical therapies, it has been suggested that ‘treating both bacterium and poison’, activating blood flow and removing blood stasis, strengthening the body resistance to eliminate pathogenic factors, and developing Chinese drugs preparations for treatment of the shock should be considered. Based on clinical practice, the authors propose the hypothesis of ‘poison and pathogenic heat dissemination-inducing stasis’ as follows: Septic shock belongs to ‘prostration syndrome’ in Chinese medicine, which is a critical disease with a severe disorder of zangfu functions, severe exhaustion, imbalance of qi and blood and body fluid, and sudden disorder of the qi of yin-yang caused by the six exogenous pathogenic factors or other noxious factors. The pathogenesis is often related to coexistence of toxic heat and blood stasis, which can reduce the function of organs to cause both qi and yin depletion, a typical concomitant syndrome of excess and deficiency. Over-production of various pathogenic mediators of inflammation is the ‘excess’, while depression of zangfu functions is the ‘deficiency’.

Reform Reformed Shengmai Yin originates from the classical prescription Shengmai San (生脉散 Pulse-activating Powder) in Nei Wai Shang Bian Hua Luo (内外伤辨惑论 The Differentiation on Endogenous and Exogenous Diseases) by LI Dong-yuan, and functions in invigorating qi, promoting blood flow, and detoxification. This formula is composed mainly of Ren Shen (人参 Radix Ginseng) 10g, Mai Dong (麦冬 Radix Ophiopogonis) 10g, Wu Wei Zi (五味子 Fructus Schisandrae) 6g, Tao Ren (桃仁 Semen Persicae) 9g, Hong Hua (红花 Flos Carthami) 6g, Shi Chang Pu (石菖蒲 Rhizoma Acori Graminei) 6g, and Bai Hua She Cao (白花蛇舌草 Herba Hedyotis Diffusae) 12g. Ren Shen can invigorate Yuan-primary qi, restore yang, and reinforce deficiency. Mai Dong can nourish yin and promote production of body fluids. Wu Wei Zi can replenish yin and produce body fluid, and is astring to cure the collapse. The contemporary pharmacological study of Chinese medical herbs shows that an effective component of Ren Shen, Panaxoside, has a regulatory action on both humoral immunity and cell immunity, resulting in significant improvement in immunological functions. Mai Dong can elevate tolerance of the myocardium to hypoxia and increase coronary blood flow. Wu Wei Zi can improve microcirculation and strengthen myocardial contractility.⁶ Tao Ren is capable of anticoagulation and ameliorating obstruction of blood flow.⁷ Hong Hua can inhibit platelet aggregation and increase the effect of fibrinolysis and antioxidants.⁸ Bai Hua She She Cao can stimulate proliferation of the reticuloendothelial system and promote antibody formation. Shi Chang Pu is effective at inducing resuscitation and restoring consciousness.⁹ The whole recipe is aimed directly at principal pathogenesis of
septic shock, and also functions in invigorating \textit{qi}, promoting blood flow, and detoxification.

In summary, invigorating \textit{qi}, promoting blood flow and detoxification is used in Chinese medicine for treatment of the middle and late phases of septic shock. The authors found that both Reformed \textit{Shengmai Yin} and dexamethasone can protect against the endotoxin-induced hypotension, brain edema, reduced SOD activity, reduced anti-oxidative capacity, and brain injury. Reformed \textit{Shengmai Yin} was more effective than dexamethasone in inhibiting the brain lipid peroxide reaction and in elevating SOD activity to reduce oxidative stress.

REFERENCES

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