Protective effect of astragalus injection against myocardial injury in septic young rats via inhibition of JAK/STAT signal pathway and regulation of inflammation

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INTRODUCTION
Sepsis is usually a critical and serious complication in clinics. It is also an important cause of multiple organ dysfunction, multiple organ failure and death. Improvements in the understanding of the pathophysiological processes involved in sepsis have revealed that systemic inflammatory response syndrome, compensatory anti-inflammatory response syndrome, and immune paralysis are important characteristics of sepsis [1]. It was recently suggested that in the early stage of sepsis, monocytes/macrophages secrete large amounts of inflammatory factors which directly or indirectly affect myocardial cells, causing myocardial...
injury, myocardial inhibition and even cardiac dysfunction [2].

At present, the key to the treatment of sepsis is to control infection in time and maintain immune homeostasis. According to traditional Chinese medicine, sepsis belongs to the category of exogenous fever which is characterized by empty mark real syndrome [3]. It has been reported that traditional Chinese medicine can achieve good clinical efficacy in the treatment of sepsis [4]. Astragalus is a traditional Chinese medicine. Astragalus injection contains astragaloside A, astragalus total saponins and other components, with beneficial effect on qi. Moreover, it contains nourishing elements, strengthens health, eliminates pathogenic factors, nourishes the heart and dredges the pulse. Studies have found that astragalus injection can also regulate the level of inflammatory factors and improve balance between pro-inflammatory and anti-inflammatory factors [5]. The protective effect of astragalus Injection on myocardial injury in septic young rats, and its effect on the imbalance between pro-inflammation and anti-inflammation, were determined in the present study.

EXPERIMENTAL

Animals

Healthy SD rats (n = 72) were purchased from Nanjing Junke Bioengineering Co. Ltd., with production license scxk (ning) 2017-0001). The rats were 5 weeks old, and had a mean weight of 125 ± 15 g.

This research was approved by the Animal Ethical Committee of Department of Pediatric, Liaocheng Second People's Hospital, Linqing, PR China ((approval no. 2018331611)), and was done in line with "Principles of Laboratory Animal Care" [6].

Main equipment and reagents

The instruments and reagents used, and their manufacturers/suppliers (in brackets) were: paraffin sectioning machine (Shenyang Hengsong Technology Co. Ltd, model: hs-st7220); low temperature high-speed centrifuge (Shanghai Hetian Scientific Instrument Co. Ltd, model TG18G); biological microscope (Shanghai Precision Instrument Co. Ltd., model: XSP-8C); electronic balance (Beijing Zhongyi HSBC Technology Co. Ltd, model ME204E); immunohistochemical staining kit (Beijing letter Shengyuan Biomedical Technology Co. Ltd); rabbit anti-human JAK2 polyclonal antibody (Shanghai Xuanling Biotechnology Co. Ltd); rabbit anti-human STAT3 polyclonal antibody (Shanghai Hengfei Biotechnology Co. Ltd.), and astragalus injection (Zhengda Qingqingbao Pharmaceutical Co. Ltd, production batch no. 33020179, specification: 10-ml packs).

Rat grouping and treatments

To establish the model of sepsis in young rats, the young rats were fed for one week in the laboratory environment at temperature of 23 ± 3 °C and humidity of 50 ± 5 %. Three rat groups were used: sham, model, and astragalus injection groups (the drug group). In the sham-operated rats, the animals were anesthetized, opened only, closed immediately, and injected with 5 mL/kg saline in tail vein. In the model group, the cecum was ligated and punctured after anesthesia, and the abdomen was closed. After operation, saline was injected into the tail vein at a dose of 5 mL/kg. In the drug group, the cecum was ligated and punctured after anesthesia, and the abdomen was closed. After operation, the tail vein was injected with 5 ml/kg astragalus injection. All rats were sacrificed at the end of the experiment.

Therapeutic indicators

Twelve young rats were selected at 12 and 24 h after the operation. Abdominal aorta blood (3ml) was centrifuged and the serum samples were kept frozen at -80 °C in a refrigerator prior to use.

Serum cardiac troponin I (cTnI) was measured with ELISA. The myocardial tissues of the young rats were isolated, fixed in 4 % formaldehyde solution, and paraffin sections were prepared. The sections were subjected to H & E staining to observe changes in myocardial morphology.

The myocardial tissue of rats was put into an ice bath of phosphate buffer and centrifuged. The resultant supernatant was subjected to assay of the levels of IL-10 and IL-6 using ELISA. Immunohistochemistry was applied for the determination of JAK2 and STAT3 expressions in myocardial tissues of the rats at 24 h.

Statistical analysis

Quantitative data were compared among multiple groups using single factor and multiple sample mean, while two groups were compared using independent sample t-test. All statistical analyses were performed with SPSS version 21.0 software. Differences were considered statistically significant at $p < 0.05$. 

Results

Serum cTn I levels

As shown in Table 1, serum cTn I level in model rats was markedly elevated, relative to sham rats, and serum cTn I was lower in the drug group than in model group (p < 0.05).

Table 1: Serum cTn I levels (mean ± SD, n = 12)

<table>
<thead>
<tr>
<th>Group</th>
<th>cTn I pg/L (12h)</th>
<th>cTn I pg/L (24h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>196.06 ± 32.34</td>
<td>205.12 ± 36.92</td>
</tr>
<tr>
<td>Model</td>
<td>494.44 ± 67.03a</td>
<td>821.62 ± 88.35a</td>
</tr>
<tr>
<td>Drug</td>
<td>415.23 ± 51.29ab</td>
<td>713.73 ± 89.63ab</td>
</tr>
<tr>
<td>F</td>
<td>105.27</td>
<td>226.85</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*P < 0.05, vs sham; **p < 0.05, vs model

Morphological changes in myocardial tissues of young rats

Myocardial tissue of sham group was normal in shape, closely arranged and without inflammatory infiltration. In the model group, the myocardial tissue was disordered, abnormal in shape and necrotic, especially after 24 h. However, lesions in the myocardial tissue of the drug group were significantly lower than those in the model group. These results are shown in Figure 1.

Levels of IL-10 and IL-6 in myocardium of young rats

Table 2 shows marked increases in the levels of IL-10 and IL-6 in model rats, relative to the corresponding levels in sham rats. Myocardial IL-10 level in the myocardial tissue of the drug-treated rats was markedly increased, while IL-6 level was markedly decreased, relative to model rats (p < 0.05).

Table 2: Concentrations of IL-10 and IL-6 in myocardium amongst the groups (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>IL-10 (ng/L) 12h</th>
<th>IL-10 (ng/L) 24h</th>
<th>IL-6 (ng/L) 12h</th>
<th>IL-6 (ng/L) 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>7.73 ± 1.15</td>
<td>8.17 ± 1.59</td>
<td>127.38 ± 21.54</td>
<td>139.11 ± 34.66</td>
</tr>
<tr>
<td>Model</td>
<td>17.48 ± 2.18a</td>
<td>27.08 ± 3.14a</td>
<td>408.78 ± 75.01a</td>
<td>596.96 ± 79.03a</td>
</tr>
<tr>
<td>Drug</td>
<td>22.98 ± 2.77ab</td>
<td>34.58 ± 3.95ab</td>
<td>325.43 ± 66.36ab</td>
<td>447.04 ± 86.58ab</td>
</tr>
<tr>
<td>F</td>
<td>156.19</td>
<td>238.22</td>
<td>71.67</td>
<td>131.27</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*P < 0.05, vs sham; **p < 0.05, vs model

Protein expressions of JAK2 and STAT3 in myocardium of young rats 24 h

Compared with the sham-operated rats, myocardial JAK2 and STAT3 proteins were upregulated in model rats. However, drug treatment markedly reduced JAK2 and STAT3 protein levels (p < 0.05). These results are depicted in Table 3.

Table 3: Protein expressions of JAK2 and STAT3 in myocardium at 24 h (n = 12)

<table>
<thead>
<tr>
<th>Group</th>
<th>JAK2 (pg/L)</th>
<th>STAT3 (pg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>5.88 ± 1.38</td>
<td>5.06 ± 0.98</td>
</tr>
<tr>
<td>Model</td>
<td>74.88 ± 7.52a</td>
<td>46.14 ± 5.81a</td>
</tr>
<tr>
<td>Drug</td>
<td>57.73 ± 6.55ab</td>
<td>36.05 ± 4.54ab</td>
</tr>
<tr>
<td>F</td>
<td>458.39</td>
<td>298.19</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

DISCUSSION

Sepsis is one of the main causes of death in critically-ill patients, and its treatment has always been a major issue in clinical medicine. Statistics have shown that in the United States, more than 800,000 people suffer from sepsis every year, out of which more than 220000 die. Infection and systemic inflammatory reactions are major manifestations of sepsis. Due to the immature development of physiological functions of various
systems and organs, and low immunity, sepsis in children has become a serious death-related disease in pediatric intensive care units [7].

Sprague-Dawley rats are the first choice for use as animal models of human diseases because of their high degree of similarity with humans with respect to metabolism and diseases, as well as their ease of reproduction. Astragalus injection is a modern Chinese patent medicine which regulates immunity and antioxidant status, and also delays ageing. It has been reported that astragalus injection improves ventricular systolic and diastolic function, and mitigates immune disorders [9]. The present study investigated the influence of astragalus injection on myocardial injury in septic young rats, and the mechanisms involved. One of the cardiac regulatory proteins is CTnI. Under normal conditions, serum cTnl level is low, but when myocardial injury occurs, serum cTnl level is significantly increased. CTnI has a high specificity for myocardial injury, and it is one of the indicators that reflect the degree of myocardial injury with high sensitivity and specificity [9]. The results of this study showed that sepsis caused myocardial damage in young rats, and that astragalus injection exerted protective effect against myocardial damage in the septic young rats.

Some of the core pathological changes in sepsis involve triggering, enhancement and amplification of inflammatory cytokines which are important in the occurrence of sepsis. Under normal conditions, pro-inflammatory and anti-inflammatory cytokines are in a dynamic balance at very low levels, thereby ensuring homeostasis. However, when sepsis occurs, a large number of pro-inflammatory and anti-inflammatory factors are released, leading to imbalance between the two factors, organ dysfunction and inflammatory damage to tissue cells [10,11].

It is known that IL-6 is one of the endogenous pyrogen which is vital for the maturation of B cells and the induction of acute phase reactive protein. Studies have found that when sepsis occurs, the level of IL-6 is significantly increased. Moreover, IL-6 can be used as a marker of inflammatory response [12]. On the other hand, IL-10 is an endogenous anti-inflammatory factor and an anti-inflammatory cytokine (with important immune regulatory function) which inhibits the expressions of inflammatory cytokines through macrophages [13].

In this study, compared with the model group, IL-10 level in the myocardial tissue of the drug group was significantly high, while the concentration of IL-6 was markedly low, and the levels of IL-10 and IL-6 in model rats were significantly higher than the corresponding levels in sham rats. These findings suggest that astragalus injection inhibits the expression of IL-6, upregulates the expression of IL-10, and improves imbalance between pro-inflammatory and anti-inflammatory factors. It has been reported that the JAK kinase/signal transducer and activator of transcription (JAK/STAT) signal pathway are involved in the pathophysiology of sepsis, and are also key pathways for a variety of cytokines [14]. The JAK is a non-receptor tyrosine protein which catalyzes tyrosine phosphorylation in cytokine receptor. Moreover, STAT is an activator of transcription. It has been reported that activated STAT enters the nucleus and activates the corresponding target genes, thereby promoting the transcription of mRNA [15]. In this study, compared with the sham operation group, the JAK2 and STAT3 protein expressions in model rats were markedly enhanced, while the protein expressions of JAK2 and STAT3 in drug-treated rats were significantly smaller than the corresponding levels in model rats. These findings suggest that astragalus injection significantly reduces the protein expressions of JAK2 and STAT3 in rat lung tissue, and inhibits the activation of JAK/STAT pathway.

CONCLUSION

Astragalus injection upregulates the expressions of IL-10 and IL-6 by inhibiting JAK/STAT signal pathway and the expression of IL-6, and reversal of the imbalance between pro-inflammatory and anti-inflammatory factors. Thus, astragalus exerts protective effect against myocardial injury in sepsis, and may be useful in the therapeutic management of myocardial infarction.
DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication. Bin Xu conceived and designed the study, Zhihong Zhao, Xuejing Wang, Xiumin Li, Hongshuang Li, Bin Xu collected and analysed the data and Zhihong Zhao wrote the manuscript.

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REFERENCES