

## Original Article

# Effect of xingnaojing injection on myocardial mitochondrial oxidative stress injury in sepsis mice

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**Abstract:** Objective: To observe the effect of Xingnaojing injection on myocardial mitochondrial oxidative stress injury in sepsis mice. Methods: The septic model were set up by receiving endotoxin by interaperitoneal injection, After consulting Xingnaojing injection by gastric tube, seventy mice were randomly divided into 7 groups, each group of ten. For respective, group LPS-6 h, group LPS-24 h, group LPS-48 h; group XNJ-6 h, group XNJ-24 h, group XNJ-48 h; control group. The myocardial mitochondrial changes, the semi-quantitative scores and the level of SOD, MDA, NO, iNOS in sepsis mice were observed. Results: Xingnaojing injection could improve the myocardial mitochondrial changes and reduce the semi-quantitative scores, significantly reduce the level of MDA, NO, iNOS and elevate the level of SOD. Conclusions Xingnaojing injection could significantly reduce the myocardial mitochondrial oxidative stress level and improve the ability of clearing oxygen radicals, thereby protect the myocardial mitochondrion in sepsis mice.

**Keywords:** Xingnaojing injection, sepsis, SOD, MDA, NO, Inos

## Introduction

Heart, as the power organ of blood circulation, is one of the target organs of septic injury. In clinic, 40~50% patients with sepsis combined with cardiac insufficiency, 7% of them were with serious heart failure [1]. Myocardial mitochondria are important injury target of sepsis. The dysfunction of myocardial mitochondria is one of the reasons leading to myocardial depressed. Many researches showed that a lot of oxygen-free radicals were generated in cardiac tissue after being infected with sepsis. The oxygen free radicals would oxidize the membrane proteins and lipids of mitochondria, and damage the integrity of myocardial mitochondrial membrane, thus suppress the cardiac function. The oxidative stress level of specifically inhibition could improve the cardiac function in rats with sepsis, finally improve prognosis [2]. Therefore, the oxidative stress level of specifically inhibition was important for protection on myocardial mitochondria in the patients with sepsis. Searching for relative medicines is one of the targets for treating sepsis. Previous study [3] showed that Xingnaojing injection (XNJ)

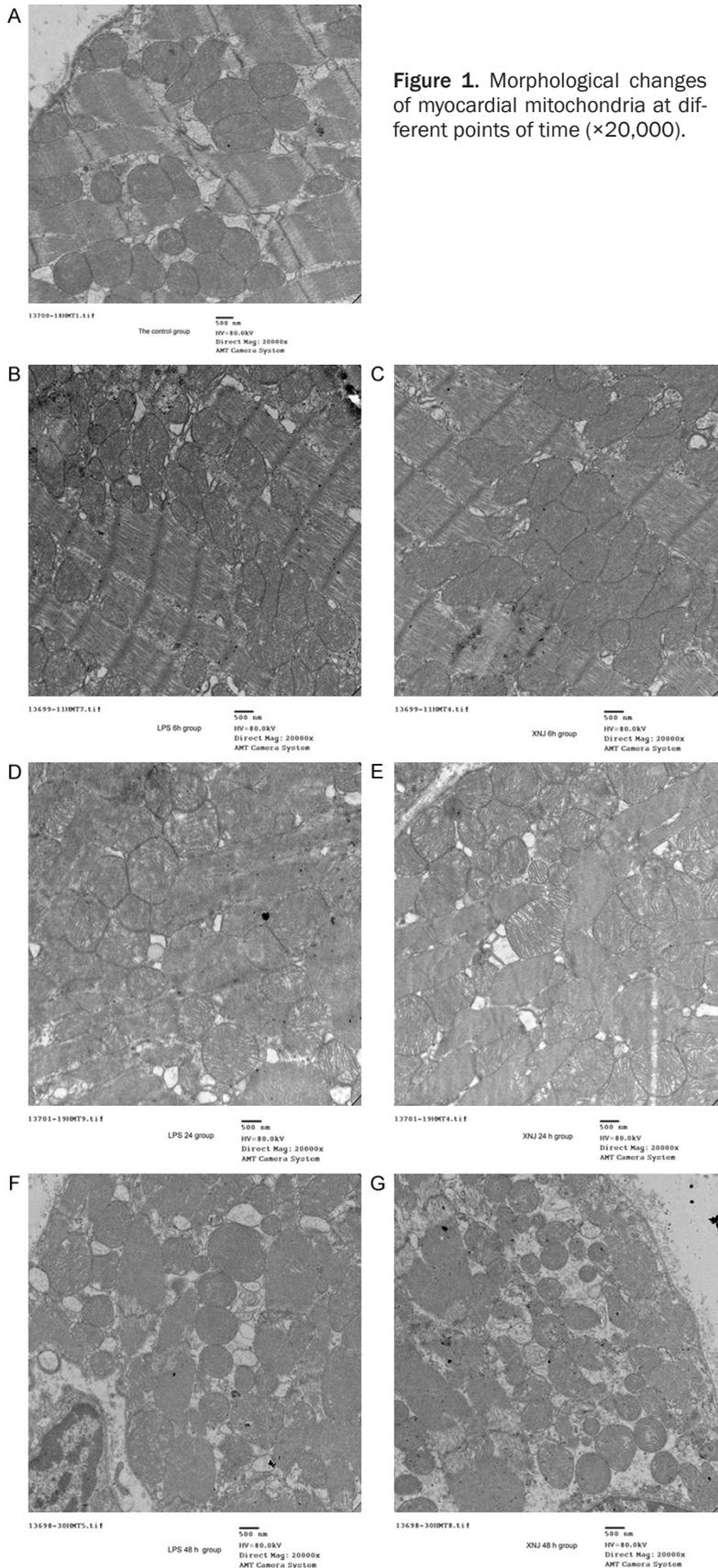
improved the systemic inflammatory response syndrome in rats induced by endotoxin, and decreased the myocardial enzyme level in rats with sepsis [4]. Therefore, we presumed that the XNJ injection had effect on the myocardial injury in patients with sepsis, but without clear mechanism. In our study, by observing the morphological changes of myocardial mitochondria in rats with sepsis under the electron microscope after injecting with XNJ injection, scoring by semi-quantitation, and detecting the oxidative stress parameters, we investigated the effect of XNJ injection on injury of myocardial mitochondria in rats with sepsis and provided the detailed mechanism of XNJ injection on injury of myocardial mitochondria with evidences.

## Materials and methods

### Subjects

Male SD rats, SPF grade, weight 220-250 g, were obtained from Medical Laboratory Animal Center of Guangdong Province, China. After feeding for one week, the rats were prepar-

## Effect of xingnaojing injection



ed for model construction, with fasting for 6 h but free drinking.

### Medicines and agents

LPS was obtained from Sigma (USA), prepared with sterile water (1 mg/mL). XNJ injection was obtained from JIMIN KEXIN SHANGHE pharmaceutical Co., Ltd, Wuxi, China (Z320205-63). SOD, MDA, NO and iNOS detection kit were obtained from Nanjing Jiancheng Bioengineering Institute, China.

### Groups and administration

70 SD rats were divided into 7 groups, randomly, 10 rats in each group. LPS groups included LPS 6 h group, LPS 24 h group, LPS 48 h group; XNJ groups included XNJ 6 h group, XNJ 24 h group, XNJ 48 h group and control group. All the rats in both LPS groups and XNJ groups were induced with LPS to construct LPS model. LPS groups: 10 mg/kg of intraperitoneal injection [5]. XNJ groups: as a result of conversion, the equivalent dose of XNJ injecting into rats by gastric tube influx was 6.4 mL/kg according to 20 mL of XNJ for each person in each day, clinically [6]. Control group: intraperitoneally injected with saline equally to the volume of LPS.

### Analysis on morphological changes and score of semi-quantitation at different points of time

We took the myocardium at different points of time, cut

## Effect of xingnaojing injection

**Table 1.** Semi-quantitation scores of myocardial mitochondria at different points of time

| Groups     | Time | Semi-quantitation scores   |
|------------|------|----------------------------|
| Control    |      | 0.024±0.015                |
| LPS groups | 6 h  | 0.399±0.042 <sup>Δ</sup>   |
|            | 24 h | 0.929±0.103 <sup>Δ</sup>   |
|            | 48 h | 0.864±0.036 <sup>Δ</sup>   |
| XNJ groups | 6 h  | 0.283±0.049 <sup>Δ</sup>   |
|            | 24 h | 0.695±0.058 <sup>Δ,*</sup> |
|            | 48 h | 0.639±0.091 <sup>Δ,*</sup> |
| F          |      | 106.517                    |
| P          |      | 0.000                      |

Note: <sup>Δ</sup>Comparing to control group, P<0.01; \*Comparing to LPS group, P<0.05.

into tubes (1 mm<sup>3</sup>), fixed in 3% glutaraldehyde and sent to detect under electron microscope in General Hospital of Guangzhou Military Command of PLA. We observed the myocardial mitochondria and scored them by semi-quantitation according to Flameng Grading Methods [7].

### Detection of SOD, MDA, NO and iNOS

Took the myocardium at different points of time, extracted the myocardial mitochondria, resuspended in the mitochondrial dissolution buffer, sonicated by ultrasound at 4°C for 5 min. After the lysis of myocardial mitochondria, the mitochondrial proteins and the level of SOD, MDA, NO and iNOS were detected.

### Statistical analysis

All the data were analyzed by SPSS 16.0 software and represented as mean ± standard deviation ( $\bar{x} \pm s$ ). After normality test and homogeneity of variance, comparisons between groups were analyzed by two-independent-sample t test. Multiple comparisons were analyzed by variance analysis. A value of P<0.05 considered significant difference.

## Results

### General conditions of rats

After 1 h of intraperitoneal injection with LPS, there were a series of symptoms of sepsis appeared in all rats, including the acceleration of heart rate and respiratory rate, obvious increase or decrease of the body temperature,

bad mental reaction, sleepiness, anti-feeding and anti-drinking, less activities, piloerection and so on. Comparing with the LPS groups, the conditions of the rats were improved in XNJ groups, while the rats in control group were without the symptoms mentioned above.

### Comparison of the morphological changes and semi-quantitation score in myocardial mitochondria at different points of time

Under electron microscope, the morphology of myocardial mitochondria in control group was approximately normal, with clear boundary, uniform matrix, compactly mitochondrial cristae, completely myocardial cell membrane, clearly intercellular space, well-arranged myofilaments. Comparing with control group, there were a few of slightly swollen mitochondria, unclear mitochondrial cristae, complete endometrium and outer membrane, without crushing mitochondrial in LPS 6 h group. In LPS 24 h group, there were a few of swollen mitochondria with vacuoles, disappearance of mitochondrial crista, unclear endometrium and outer membrane, parts of the myocardial fiber breaking. In LPS 48 h group, there were different degrees of swollen mitochondria with vacuoles, the decreasing or solidifying density of matrix, breaking mitochondrial cristae and parts of the myocardial fiber. Comparing with LPS groups, the morphology of myocardial mitochondria was improved in XNJ groups (Figure 1A-G).

The results of semi-quantitation: comparing with control group, the scores of LPS groups and XNJ groups increased significantly (P<0.01). In LPS groups and XNJ groups, the scores increased gradually, and reached the peak at 24 h, then decreased gradually. The scores of 24 h groups were higher than 6 h (P<0.01). But comparing to 48 h group, there were no significant differences (P>0.05). In 24 h groups and 48 h groups, the scores of XNJ groups were lower than LPS groups (P<0.05), while there were no significant differences at 6 h between the two (P>0.05) (Table 1).

### SOD, MDA, NO and iNOS level at different points of time

Comparing with control group, SOD, MDA and iNOS level increased in LPS groups and XNJ groups, significantly (P<0.05). In LPS groups and XNJ groups, SOD, MDA and iNOS increased

## Effect of xingnaojing injection

**Table 2.** Comparing of SOD, MDA, NO and iNOS level at different points of time

| Groups     | Time | SOD                       | MDA                      | NO         | iNOS                     |
|------------|------|---------------------------|--------------------------|------------|--------------------------|
| Control    |      | 3.35±0.87                 | 1.32±0.25                | 16.92±0.80 | 2.83±1.35                |
| LPS groups | 6 h  | 7.39±1.65 <sup>Δ</sup>    | 2.34±0.42 <sup>Δ</sup>   | 16.95±7.27 | 3.24±0.23                |
|            | 24 h | 12.32±2.61 <sup>Δ</sup>   | 4.33±0.57 <sup>Δ</sup>   | 19.32±5.80 | 5.64±1.05 <sup>Δ</sup>   |
|            | 48 h | 9.85±2.10 <sup>Δ</sup>    | 2.71±0.46 <sup>Δ</sup>   | 19.79±6.28 | 4.86±1.36 <sup>Δ</sup>   |
| XNJ groups | 6 h  | 9.11±1.06 <sup>Δ*</sup>   | 1.99±0.39 <sup>Δ</sup>   | 16.26±6.44 | 3.05±0.53                |
|            | 24 h | 16.27±1.27 <sup>Δ,*</sup> | 3.02±0.21 <sup>Δ,*</sup> | 16.63±4.46 | 3.61±0.81 <sup>Δ,*</sup> |
|            | 48 h | 10.69±1.34 <sup>Δ</sup>   | 2.32±0.46 <sup>Δ</sup>   | 17.14±3.09 | 4.12±0.94 <sup>Δ</sup>   |
| F          |      | 17.917                    | 21.018                   | 0.408      | 4.113                    |
| P          |      | 0.000                     | 0.000                    | 0.869      | 0.014                    |

Note: <sup>Δ</sup>Comparing to control group, P<0.01; \*Comparing to LPS group, P<0.05.

gradually, and reached the peak at 24 h, then decreased gradually. In LPS groups, SOD, MDA and iNOS level in 24 h group were significantly higher than in 6 h group (P<0.05), while MDA level was significantly higher than in 48 h group (P<0.05), and SOD, iNOS level were without significant differences comparing to 48 h group (P>0.05). In XNJ groups, SOD and MDA level in 24 h group were significantly higher than in 6 h group and 48 h group (P<0.05), while there were no significant differences of iNOS comparing to 6 h group and 48 h group (P>0.05). In 6 h group and 24 h group, SOD level in XNJ groups was higher than in LPS groups, significantly (P<0.05). In 24 h group, MDA and iNOS level in XNJ groups were lower than in LPS groups, significantly (P<0.05). Comparing to LPS groups, NO content decreased at different points of time in XNJ groups, but without significant differences (P>0.05) (Table 2).

### Discussion

Sepsis is a kind of systemic inflammatory response syndrome induced by infection. The cause of the disease is that the over-release of cytokines and inflammatory mediators during the body infection induced by immunogenic materials, which leads to the out of control of systemic inflammatory response and immune dysfunction. This pathophysiological process leads to autoimmune injury in the host, which is with the highest mortality in the ICU. When with sepsis, bacterial toxin and cytokines could induce the inadequate perfusion of myocardium, myocardial energy metabolic dysfunction, myocardial apoptosis and so on by different pathways, which leads to

cardiac dysfunction [8]. The mortality of the patients with sepsis companied with serious cardiac dysfunction (70%) is 50% higher than those with sepsis but without serous cardiac dysfunction (20%) [9]. Therefore, the improvement of cardiac function in the patients with sepsis is important to improve the prognosis.

The mechanism of sepsis with cardiac function is complicated, including mi-

tochondrial dysfunction, damage of cytokines and inflammatory mediators, imbalance of calcium homeostasis, apoptosis, circulation and microcirculation change and so on. Among them, myocardial mitochondria are important damaged targets, with the myocardial mitochondrial dysfunction as one of the important causes in myocardial depressant [10]. Many researches showed that after being infected with sepsis, there were lots of oxygen free radicals generating in myocardium, which would oxidize mitochondrial membrane proteins and lipids, damage the integrity of myocardial mitochondria membrane, thus influence cardiac function [2]. In our study, we found that there were mitochondria swelling with vacuoles, mitochondrial cristae disappearing, unclear endometrium and outer membrane and so on. Comparing to control group, the scores of semi-quantitation in LPS groups and XNJ groups increased significantly, the same to the level of MDA and iNOS, while NO level did not increase significantly, which indicated that there were lots of oxygen free radicals of oxidative stress status generating in the myocardial mitochondria in rats with sepsis. Otherwise, the SOD increased in the myocardial mitochondria of the rats with sepsis, which was with the ability to clean the oxygen free radicals. This phenomenon indicated that there was regulating capacity in the body with early sepsis. Those changes mentioned above appeared in the early stage of sepsis, which indicated that the myocardial mitochondria were damaged in the early stage of sepsis.

XNJ injection was extracted from the traditional Chinese medicine "Angong Niu Huangwan" and

made into water-soluble intravenous injection. XNJ injection, as the Chinese traditional compound medicine and with the abilities of heat-clearing and detoxifying, cooling and activating blood and inducing resuscitation, was mainly composed by musk, borneol, curcuma, cape jasmine and so on [11]. Some researchers found that XNJ injection could improve the systemic inflammatory response syndrome induced by sepsis in rats by suppressing the activity of NF- $\kappa$ B and the level of IL-6 (3). Otherwise, XNJ injection could reduce the CK and CK-MB level in the serum of rats with sepsis, inhibit the expression of TNF- $\alpha$  in myocardium, and regulate the NF- $\kappa$ B signal transduction pathway, then suppress the inflammatory and immune response in sepsis to improve the myocardial injury [4]. We presumed that XNJ injection had the effect on the myocardial injury of sepsis, but without clear mechanism. In our study, XNJ could regulate the morphological changes and decrease the scores of semi-quantitation in myocardial mitochondria. Additionally, XNJ could significantly reduce the level of MDA, NO and iNOS and increase SOD level in myocardial mitochondria in rats with sepsis. The action time of XNJ was mainly at 6 h and 24 h. After 24 h, there was no obvious effect, which might be related to drug metabolism. This phenomenon indicated that XNJ could protect the myocardial mitochondria in rats with sepsis at the early stage.

To draw a conclusion, the myocardial mitochondria in rats with sepsis were damaged from the early stage. There were lots of oxygen free radicals generating in myocardial mitochondria and were in oxidative stress status. We firstly discovered that XNJ could reduce the oxidative stress level of myocardial mitochondria and improve the ability of cleaning the oxygen free radicals to protect the myocardial mitochondria in rats with sepsis. However, in our study on the cardiac function in rats with sepsis, we did not find the mechanism of XNJ on how to finally improve the cardiac function and reduce the oxidative stress level of myocardial mitochondria. We still need further studies to investigate the mechanism observe the effect of XNJ injection.

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### Disclosure of conflict of interest

None.

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