Observation of changes in the number of myocardial capillaries in rabbits after treatment of acute myocardial infarction by Tongxinluo superfine powder

Wu Haotian, Zhang Fengshun, Liu Jinyi, Li Xuezhao, Guo Fei, Wu Yiling

RESULTS: In the treatment and pre-treatment groups, ejection fraction, left ventricular short axis shortening rate, left ventricular end-systolic diameter and cardiac output significantly improved, the number of capillaries significantly increased, and infarct size significantly decreased. In addition, the results suggest that the value of intra-ventricular pressure and the situation of electrocardiogram also changed to different degrees with the increasing of treatment of cycle.

CONCLUSION: Tongxinluo superfine powder can protect the myocardium, improve the blood supply of the myocardium and reduce the degree of myocardial injury, during acute stage of myocardial infarction.

© 2018 JTCM. All rights reserved.

Keywords: Myocardial infarction; Myocardium; Microcirculation; X-Ray Microtomography; Ferric chloride; Tongxinluo superfine powder

INTRODUCTION

Acute myocardial infarction (AMI) is caused by acute myocardial ischemia. The major pathological basis of most AMI is coronary artery thrombosis induced by unstable atherosclerotic plaque rupture or erosion. Since 1987, the annual rate of hospitalization for AMI and fatal coronary artery disease has increased by 4%-5% in the United States. 550,000 patients suffer AMI for the first time and 200,000 recurrent patients are still reported each year. On a global scale, ischemic heart disease has become the leading cause of death. However, its prevalence has gradually shifted to developing countries, and accounts for more than 80% of...
death population of cardiovascular disease worldwide. With the improvement in living standards and the deterioration of dietary habits and environmental factors, the incidence of cardiovascular disease in China has increased year by year, becoming one of the most serious health problems, and this has caused huge economic and social burdens. Atherosclerosis is the pathological basis for cardiovascular and cerebrovascular diseases. Its progression, from the early formation of a plaque to the blockage of blood vessels and the triggering of myocardial infarction, must undergo a long period time of pathological changes. Therefore, targeted early prevention is particularly critical.

A biology experiment was once designed based on the laws of Fluid physics. The external application of ferric chloride solution can induce the separation and (make the junctions of vascular endothelial cells separated) to expose collagen, cause platelet adhesion and aggregation, and finally activate the coagulation system to form mixed thrombi. Ferric chloride can cause vascular intima and smooth muscle damage, where iron ions enter the vascular lumen through endocytosis-exocytosis, induce endothelial injury through an oxidation effect, cause platelet activation, adhesion and aggregation, and eventually induce thrombosis. The method is simple, easy to use and repeatable, and the morphological features induced by this method are close to those of an autogenous thrombus. Therefore, it is of great significance to analyze the pathogenesis of thrombosis, as well as for the research and development of anticoagulant and thrombolytic drugs, to improve blood-flow has important sense.

Tongxinluo superfine powder is composed of 12 kinds of Chinese herbs, including a variety of plants and insect drugs; it is prepared into a micron or nanometer powder using superfine grinding technology, to increase the dissolution rate of the active ingredients, greatly improving its bioavailability. In recent years, some studies have revealed that Tongxinluo can improve the internal material and information circulation. This could further stabilize atherosclerotic plaques, protect against reperfusion injury after AMI, and maintain the integrity of the microvascular structure and function, as well as protect endothelial cells. Therefore, Tongxinluo has anti-inflammation, anti-oxidation, and anti-apoptosis effects and improves microcirculation. Conventional therapies for AMI, such as thrombolysis, interventional revascularization and surgical treatment exert certain effects in improving the quality of life and long-term prognosis of patients. However, the situation is not fundamentally solved by these treatments. Therefore, to solve the problem in the present study, we first establish a rabbit model of AMI by externally applying ferric chloride to the circumflex branch of the left coronary artery, and to further research the biological mechanism of Tongxinluo superfine powder on AMI was investigated by using echocardiography, micro-computed tomography (microCT) and histopathological staining, to further provide a theoretical basis for the treatment of AMI by Tongxinluo.

**MATERIALS AND METHODS**

**Experimental animals**
A total of 32 healthy New Zealand white rabbits with an average body weight of 2.5-3.0 kg (both male and female) were used for this study. All rabbits were provided by the Beijing Xinglong Experimental Animal Breeding Farm, and fed in an ordinary environment (on the conditions of normal diet and drinking instruments and reagents: MICROFIL (Flow Tech. Inc., UM, USA), ferric chloride (Hengxing Chemical Reagent Co., Ltd., NKU, Tianjin, China), ethyl carbamate (Shanpu Chemical Co., Ltd., FUDAN, Shanghai, China), penicillin (North China Pharmaceutical Co., Ltd. HBU, Baoding, China), Vivid E9 color Doppler ultrasound scanner (GE, NK, USA), Quantum GX small animal microCT (PerkinElmer, NJ, Paterson, USA), animal electrocardiogram analysis system (Softron Biotechnology Co., Ltd., PKU, Beijing, China), and a MP150 polygraph physiological signal recorder (Biopac, NV, RENO, USA).

**Tongxinluo superfine powder**
The powder (the herbs which could improve the functions of circulation) was prepared an ultra-fine pulverization technology, and the average grain diameter was less than 10 nm. This particle size can achieve cell penetration, which is advantageous in enhancing the efficacy of the drug action.

**Grouping and medication**
A total of 32 New Zealand white rabbits were randomly divided into four groups: sham operation group, model group, treatment group and pre-treatment group (n = 8). In the sham operation group, external application of ferric chloride was not performed on the circumflex branch of the coronary artery, while the remaining steps were the same as those in the other three groups. In the treatment group, 0.125 g/kg of Tongxinluo superfine powder was used for gavage once daily for 7 d from the first day after the operation. In the pre-treatment group, gavage was performed 3 d before the operation, and the procedures and doses were the same as those in the treatment group. In the sham operation group and model group, gavage was performed using the same dose of normal saline daily.

**Establishment of the animal model**
Rabbits were weighed, injected using 4 mL/kg of 25% ethyl carbamate at the marginal vein of the ear, fixed, and the skin on the chest was shaved. Routine disinfection was performed, and a hole-towel was placed. The skin, fascia and muscles were cut off layer by layer, the 2nd and 3rd ribs were amputated along the left edge of the 3rd ribs were amputated along the left edge of the 3rd ribs were amputated along the left edge of
the sternum, and the chest was opened using a chest-wall distractor to expose the heart. The pericardium was cut off, and the circumflex branch of the left coronary artery was located. A cotton swab previously dipped in 70% ferric chloride solution was placed in the middle and lower segment of the circumflex branch for 30 min. The establishment of the model was successful when the electrocardiogram revealed a significant elevation in the ST segment. The swab was taken out, and the muscles and skin were sutured layer by layer. Then, the rabbits were injected using 800 000 units of penicillin in the left leg muscle once daily for 3 d, to prevent incision infections.

Echocardiography
Rabbits in each group were scanned using a GE Vivid E9 color Doppler scanner. Scanning conditions: 2SS-D heart probe, at a frequency of 12 MHz; scanning was performed from the short axis angle of the right sternum. The morphological and functional parameters of left ventricular inter diameter at end-diastole (LVIDd), left ventricular internal diameter Systole (LVIDs), ejection fraction (EF), fractional shortening (FS) and stroke volume (SV) were measured according to the criteria of the American Society of Echocardiography.\(^1\)

Acquisition and analysis of the electrocardiogram
After anesthesia, rabbits in each group were connected to the animal electrocardiogram analysis system, electrocardiograms were acquired, and changes in the electrocardiogram were monitored during the operation. The electrocardiograms were acquired again before the animals were sacrificed 1 weeks later. Then, the electrocardiogram data acquired at the three time points were statistically analyzed using the animal electrocardiogram analysis system.

Left ventricular internal pressure measurements
Rabbits in each group were anesthetized (4 mL/kg of 25% urethane), fixed and shaved. Then, routine cervical disinfection was performed, cervical surgical drapes were placed, an incision was made at the median neck to expose the right common carotid artery, and the pressure probes were connected to the polygraph physiological signal recorder. After pressure calibration, the common carotid artery catheter was inserted into the left ventricle. The left ventricular internal pressure was recorded.

Measurement of infarct size
From each group, four rabbits were randomly selected for histological analysis. An incision was made at the left sternal margin to expose the heart, and the rabbit was sacrificed through injection of 10% KCl at the marginal vein of the ear. The heart was quickly collected, rinsed using 0.9% saline, fixed using 10% neutral formalin, and stained using hematoxylin, eosin and Masson staining, according to standard procedures. The infarct size ratio and fibrotic size ratio were calculated using Image-Pro-Plus 6 software (Media Cybernetics, Inc., UCLA, USA); that is, infarct size/total area, and fibrotic size/total area.

Cardiac MicroCT scan and 3D reconstruction
Four rabbits were randomly selected from each group to undergo MicroCT analysis after echocardiography and electrocardiogram measurements. After routine anesthesia, fixation and disinfection, the rabbits were injected using heparin (3 mL, 1000 IU/mL) at the marginal vein of the ear for systemic heparinization, and were sacrificed through bolus injection of 10% KCl. The chest was opened through the left sternal margin approach. Then, ascending aorta intubation was performed, the heart was rinsed using normal saline, and coronary perfusion was performed using MICROFIL (MV120 bulk solution/diluting agent/hardening agent = 8 : 1 : 1) at a constant pressure of 100 mm Hg until it flowed into the blood vessels of the myocardium through the coronary artery, remained in the vein, but did not flow outward. Finally, the heart was taken out, placed in 10% formalin, and placed in a refrigerator at 4 °C for 24 h to ensure that the MICROFIL had solidified.

A whole-heart scan was performed using the Quantum GX small animal MicroCT. Scanning conditions: FOV (field of view) = 72 mm, power = 8 W, and voltage = 90 kV. The scanned images were processed using Living Imaging 4.5.2 software (PerkinElmer). The morphological data of the coronary artery tree were extracted from the MicroCT images using the grayscale threshold method.\(^1\)

Statistical analysis
Statistical differences among groups were evaluated a two-way ANOVA (Sigma Stat 3.5), was used to detect the stistical difference of morphometric parameters between treatment and pre-treatment groups (offered by ISI of PKU, Beijing China). Data were presented as mean ± standard deviation. The date of each group were summarized in the above study, including the infarct size after cardiac 3 d reduction, and routine date changes after intervention treatment. A \(P < 0.05\) was considered to indicate statistical significance.

RESULTS
Echocardiographic results
After treatment by using Tongxinluo superfine powder, make a comparison between the results of the treated animals and sham operation group. The ejection fraction, left ventricular short axis shortening rate, left ventricular end-systolic diameter and cardiac output significantly improved in the treatment group and pre-treatment group \(P < 0.05\). The differences in left ventricular end-diastolic diameter among these four groups were not statistically significant \(P > 0.05,\) Table 1.
end-systolic pressure was significantly lower ($P < 0.05$) compared with the sham, treatment group, and pre-treatment group. Left ventricular end-diastolic pressure significantly increased ($P < 0.05$) in the model group (Table 2). This demonstrates that Tongxinluo superfine powder can significantly improve the systolic function of the heart.

Analysis of 3D vascular images and 3D reconfiguration of capillaries was significantly increased in the treatment group and pre-treatment group when compared with the model group and sham operation group ($P < 0.05$). Figure 2 presents the pathological staining results of the rabbit cardiac tissues in each group. The infarct size significantly decreased in the treatment group and pre-treatment group compared with the model group ($P < 0.05$). The above results reveal that Tongxinluo superfine powder can induce an obvious increase in myocardial capillaries, improve the blood supply of the myocardium, and reduce the size of a myocardial infarction.

**DISCUSSION**

In the current study, under the inspiration of Traditional Chinese Medicine, and on the basis of a classical prescription, a prescription was tested consisting of Chinese herbal medicine, including ginseng, red peony root, lignum acronychiae, frankincense, leech, scorpion, centipede, periostracum cicada, steleophaga, Semen Zizyphispinosae and borneol. Intervention using this prescription can improve blood circulation. In recent years, more studies have revealed that research based on the pathogenesis of atherosclerosis, the characteristics of insect drugs, such as scorpion, centipede and periostracum cicada, or leech and steleophaga, or leech and scorpion, has lend themselves to atherosclerosis treatment. This prescription has special effects in controlling cardiovascular and cerebrovascular diseases and has anticancer properties. In the early stages of AMI, levels of oxygen-free radicals increase in the body, while the activity of enzymes (such as superoxide dismutase) resistant to oxygen-free radicals decreases. This leads to the accumulation of oxygen-based pathological substances that react with unsaturated fatty acids in biological membranes [intracellular lactate dehydrogenase (LDH) and creatine kinase (CK)] are released into the blood, which causes the activity of blood

### Table 1 Echocardiographic indexes of rabbits in each group ($\bar{x} \pm s$)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sham ($n = 8$)</th>
<th>Model ($n = 8$)</th>
<th>Treatment ($n = 8$)</th>
<th>Pre-treatment ($n = 8$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF%</td>
<td>71.26±6.30</td>
<td>56.57±3.14</td>
<td>68.63±2.65</td>
<td>69.15±4.87</td>
</tr>
<tr>
<td>FS%</td>
<td>33.52±2.17</td>
<td>26.41±0.55</td>
<td>34.87±2.55</td>
<td>32.64±1.73</td>
</tr>
<tr>
<td>LVIDs (cm)</td>
<td>0.87±0.15</td>
<td>1.02±0.12</td>
<td>0.85±0.11</td>
<td>0.87±0.05</td>
</tr>
<tr>
<td>LVIDd (cm)</td>
<td>1.31±0.15</td>
<td>1.34±0.17</td>
<td>1.32±0.12</td>
<td>1.33±0.11</td>
</tr>
<tr>
<td>SV (mL)</td>
<td>3.17±0.41</td>
<td>2.64±0.30</td>
<td>3.02±0.27</td>
<td>3.15±0.37</td>
</tr>
</tbody>
</table>

Notes: sham operation group and model group received the same dose of normal saline daily. Treatment group treated with 0.125 g/kg of Tongxinluo superfine powder, once daily for 7 d from the first day after the operation. Pre-treatment group treated with 0.125 g/kg of Tongxinluo superfine powder 3 d before the operation, and the procedures and doses were the same as those in the treatment group.

Figure 1 presents ultrasound images demonstrating the cardiac function of rabbits in each group. The above data revealed that Tongxinluo superfine powder could improve cardiac function.

**Electrocardiogram results**

During the establishment of the model, the elevation at the ST segment in each group was significantly higher than that before the operation ($P < 0.05$). During establishment of the model, the height of the T wave in each group was significantly higher than that before the operation ($P < 0.05$). However, the postoperative electrocardiogram results revealed that the differences in the degree of elevation at the ST segment and the height of the T wave among the model group, treatment group, and pre-treatment group were not statistically significant ($P > 0.05$). Compared with the sham, treatment, and pre-treatment group, left ventricular end-systolic pressure was significantly lower ($P < 0.05$) and left ventricular end-diastolic pressure significantly increased ($P < 0.05$) in the model group (Table 2). This demonstrates that Tongxinluo superfine powder can significantly improve the systolic function of the heart.

Analysis of 3D vascular images and 3D reconfiguration of capillaries was significantly increased in the treatment group and pre-treatment group when compared with the model group and sham operation group ($P < 0.05$). Figure 2 presents the pathological staining results of the rabbit cardiac tissues in each group. The infarct size significantly decreased in the treatment group and pre-treatment group compared with the model group ($P < 0.05$). The above results reveal that Tongxinluo superfine powder can induce an obvious increase in myocardial capillaries, improve the blood supply of the myocardium, and reduce the size of a myocardial infarction.

DISCUSSION

In the current study, under the inspiration of Traditional Chinese Medicine, and on the basis of a classical prescription, a prescription was tested consisting of Chinese herbal medicine, including ginseng, red peony root, lignum acronychiae, frankincense, leech, scorpion, centipede, periostracum cicada, steleophaga, Semen Zizyphispinosae and borneol. Intervention using this prescription can improve blood circulation. In recent years, more studies have revealed that research based on the pathogenesis of atherosclerosis, the characteristics of insect drugs, such as scorpion, centipede and periostracum cicada, or leech and steleophaga, or leech and scorpion, has lend themselves to atherosclerosis treatment. This prescription has special effects in controlling cardiovascular and cerebrovascular diseases and has anticancer properties. In the early stages of AMI, levels of oxygen-free radicals increase in the body, while the activity of enzymes (such as superoxide dismutase) resistant to oxygen-free radicals decreases. This leads to the accumulation of oxygen-based pathological substances that react with unsaturated fatty acids in biological membranes [intracellular lactate dehydrogenase (LDH) and creatine kinase (CK)] are released into the blood, which causes the activity of blood
and promotes capillary proliferation, which is the core mechanism of cardiovascular regeneration.

Modern pharmacological experiments have confirmed that the Chinese herbal medicine compound Tongxinluo can improve the structure of platelets, inhibit the secretion of CD40L (TNF associated activation protein TRAP), PF4 (platelet factor 4) and sCD62P (P-Selection), reduce the cytosolic calcium concentration and inhibit its activation, and decrease the adhesion of platelets to endothelial cells. A study on ginseng, the main drug in Tongxinluo, revealed that it could not only inhibit adenosine diphosphate-induced platelet aggregation and reduce platelet surface activity, but also improve platelet ultrastructure, reduce pseudopod formation and inhibit alpha particles and dense granule release. This mechanism is related to the inhibition of phosphodiesterase activity, the enhancement of cAMP levels in platelets, and the reduction of calcium ions. In addition, Tongxinluo improves the dysfunction of vascular endothelial cells, increases serum nitric oxide levels and bioavailability, and decreases plasma endothelin, Ang II and other factors in rats. Further studies have confirmed that this prescription could improve the anti-oxidation ability of vascular endothelial cells, upregulate the expression of endothelial nitric oxide synthase and improve endothelium-dependent relaxation of HO-1 (heme oxygenase), P47-phox and P22-phox expression.

In conclusion, Tongxinluo superfine powder can improve cardiac function and myocardial contractility in a model of coronary artery thrombosis induced by ferric chloride. The main mechanism is an increased number of myocardial capillaries and a reduced size of myocardial infarction. This study has revealed that Tongxinluo superfine powder can significantly improve the acute stage of AMI and significantly improve the prognosis of AMI.

**ACKNOWLEDGEMENTS**

We are particularly grateful to all the participants of the study in college of engineering peking university and college and medicine of Hebei university.

**REFERENCES**


3 Murray CJ, Barber RM, Foreman KJ. Global, regional, and national disability-adjusted life years (DALYs) for 306 diseases and injuries and healthy life expectancy (HALE) for 188 countries, 1990-2013: quantifying the epidemiologic transition. Lancet 2015; 386(10009): 2145-2191.


