

## RESEARCH ARTICLE

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

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### Abstract

**OBJECTIVE:** To investigate the effects of Tongxinluo superfine powder on cardiac function, infarct size and the number of myocardial capillaries in a rabbit model of acute myocardial infarction.

**METHODS:** A total of 32 New Zealand white rabbits were randomly divided into four groups: sham operation group, model group, treatment group, and pre-treatment, the experiment of pre-treatment group was performed 6 weeks early than the (treat) group. The four groups use a unified modeling technique. An acute myocardial infarction model was established through external application of 70% ferric chloride on the coronary artery. After 7 d, electrocardiogram, ultrasonography of cardiac function, micro-computed tomography, pathology and other data were collected.

**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.

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**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.<sup>1</sup> 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.<sup>2</sup> On a global scale, ischemic heart disease has become the leading cause of death.<sup>3</sup> However, its prevalence has gradually shifted to developing countries, and accounts for more than 80% of

death population of cardiovascular disease worldwide.<sup>3,4</sup> 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.<sup>5</sup> 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.<sup>6,7</sup> 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.<sup>8</sup> 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.<sup>9,10</sup> 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.<sup>11-14</sup> 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

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