

# SiNiTang – A Traditional Chinese Herbal Remedy Improves Cardiac Function Post-MI

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## Abstract

SiNiTang is a well known traditional Chinese herbal remedy. It is used to treat cold extremities, weak pulse and lethargy: Symptoms that correspond with myocardial infarction and heart failure in western medicine. To examine potential beneficial effects on post-MI remodeling, a modified SiNiTang formula was tested using a well established model of myocardial infarction in Rats. Application of SiNiTang significantly reduced left ventricular dilation and thus preserved left ventricular function. However, it did not affect the hypertrophic response. Exploratory histopathological examination revealed better vascularization of infarct and peri-infarct areas in SiNiTang treated animals compared to placebo. HUVEC cells were treated with SiNiTang showed increased proliferation and tube formation. SiNiTang attenuates ventricular dilation post-MI and increases neoangiogenesis.

**Keywords:** Myocardial infarction; Post-MI remodeling; Traditional chinese medicine; Angiogenesis

**Abbreviations:** TCM: Traditional Chinese Medicine; MI: Myocardial Infarction; FS: Fractional Shortening; LAD: Left Anterior Descending Coronary Artery; LVEDD: Left Ventricular End-Diastolic Diameter; LVESD: Left Ventricular End-Systolic Diameter; HUVEC: Human umbilical Vein Endothelial Cells

## Introduction

Traditional Chinese Medicine (TCM) provides a holistic approach to health and disease. It is a complex system built on centuries of observation of disease processes. Herbal medicine is a key component within TCM. In recent years, scientific interest in Chinese herbal remedies increased. This is partly due to the hope to identify new bioactive components within used herbs [1,2]. In contrary to the reductionist approach of western medicine, Chinese herbal remedies can be used for a variety of symptoms and consist of multiple components. In contrast to the increasing interest in TCM, studies evaluating the effects of TCM remedies in standardized animal models are rare. However, applying these remedies to standardized models of disease can help to understand Chinese medicine in the context of western medicine.

Therefore we decided to evaluate a well known Chinese herbal remedy SiNiTang using a well established model of myocardial infarction (MI) in rats [3,4]. SiNiTang is composed of three ingredients (Radix Lateralis Aconiti Carmichaeli, Rhizoma Zingiberis Officinalis and Radix Glycyrrhizae Uralensis) and is according to Chinese Medicine given in conditions with cold extremities, lethargy and weak pulse [5]. The condition corresponds with myocardial infarction and heart failure in western medicine [6]. Therefore we applied this Chinese remedy on the well established rat model of chronic MI due to occlusion of the LAD. This model is commonly used to study drug effects and became popular due to its role in establishing ACE inhibitors as standard therapy post-MI [4].

## Methods

### Animals

Adult male Sprague-Dawley rats weighing 316±34g were obtained from the Core Unit for Biomedical Research of the Medical University

of Vienna, Himberg, Austria. Animals were housed with a 12/12 hour light/dark cycle in temperature controlled environment and allowed free access to food and water. All animal procedures were approved by the institutional ethics committee as well as the ethics committee for animal trials in Austria.

### Rat model of MI

MI was induced using the well established model of chronic LAD ligation [3,4]. Briefly, rats were anesthetized using a ketamine (100mg/kg i.p.) and xylazine (5mg/kg i.p.), intubated using a small polyethylene cannula and the anesthesia maintained with 1% Isoflurane. The chest was opened using a lateral thoracotomy in the 4th intercostal space. The heart was made visible and a prolene 6-0 suture was used to ligate the LAD. Development of MI was visible in ECG changes and by paleness of the myocardium distal to the ligature. The chest was closed and anesthesia stopped. Upon spontaneous breathing, rats were extubated. To reduce pain post-operatively rats were treated with piritramide (3mg s.c.). Sham operated animals (n=6) served as control. One week post-MI animals were randomized into either receiving SiNiTang, a Chinese herbal remedy (MI+T, n=10) or placebo (MI, n=10) via drinking water.

### Preparation of SiNiTang and treatment regime

Fresh dried Chinese herbs were supplied by the Shandong University of Traditional Chinese Medicine, Shandong, China. As some ingredients are potentially toxic substances according to TCM, the dosage of the ingredients varied in the course of the treatment according to Table 1. Further Cinnamomum Cassia was added as additional substance to

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	Days 7-13	Days 14-28	Days 29-35	Days 36-42
Quantity	3ml/d	3ml/d	4ml/d	5ml/d
Radix Glycyrrhizae Uralensis	25 g	25 g	30 g	30 g
Zingiberis Officinalis	10 g	10 g	12 g	15 g
Radix Lateralis Aconiti	5 g	8 g	8 g	8 g
Cinnamomum Cassia	3 g	3 g	3 g	3 g

**Table 1: Treatment Regime with SiNiTang.** Treatment with SiNiTang started randomized on day 7 post-MI. The dosages of the single components were slowly increased during the experiment.

balance the effects of the other components in long term treatment [6]. The herbs were mixed as described in Table 1. The herbal mixture was soaked in a ceramic cooker with cold water for an hour. After this, the mixture was cooked gently for 45 minutes, filtered, and the tea was stored. After adding new water, the herbs were cooked again for 45 minutes. Both teas were combined, stored until usage at 4°C and diluted to result in the quantities in Table 1 before administration. After dilution, the decoction was administered to the rats by drinking water.

To ensure constant quality of the preparations, each preparation was subjected to HPLC analysis as described before [7]. Briefly, reverse phase HPLC was performed using an endcapped C-18 stationary phase and acetonitrile as buffer. Detection was performed at 254nm.

### Echocardiography

Echocardiography was performed 6 weeks post-MI on Rats anesthetized using a mixture of Ketamine (100mg/kg i.p.) and Xylazine (5mg/kg i.p.). Ultrasound was performed using 7.5 Mhz pediatric probe attached to a digital echocardiography console (GE Vivid7). A left parasternal long axis image was obtained, followed by a left parasternal short axis image at the mid-papillary level. To measure cardiac function M-Mode images were obtained at the mid-papillary level. Fractional Shortening was calculated as  $FS = (LVEDD - LVESD) / LVEDD$ .

### Post-Mortem examination and Histopathology

Rats were sacrificed 6 weeks post-MI. Heart and bodyweight were recorded. Samples for histological examination were stored in 20% Formalin at 4°C until histological processing.

For histopathological analysis hearts were cut in three equal cross sections, embedded in paraffin and cut into 5µm thick slides. Slides were stained with Hematoxylin-Eosin for general analysis. To examine vasculature immunohistological staining for von Willebrand Factor (vWF) was performed. After deparaffinization and antigen-retrieval a rabbit-polyclonal antibody directed against vWF (Abcam) was used. A FITC conjugated secondary antibody (SIGMA) was then introduced, cover slips mounted using DAPI containing mounting medium and slides examined using a fluorescence microscope (Zeiss Imager Z3, Zeiss).

Myocardial cross sectional area was measured from slides stained with TRITC-conjugated lectin (Sigma). Slides were incubated with TRITC-conjugated lectin (10µg/ml) for 3 hours at 37°C after deparaffinization and antigen-retrieval. Slides were imaged using a fluorescence microscope and cross sectional areas measured. A total of 100 randomly located myocytes were measured per animal and the median cross sectional area was used for further analysis.

### In-Vitro HUVEC proliferation and tube formation

Human umbilical vein endothelial cells (HUVEC) were isolated

from fresh umbilical cords by mild collagenase treatment and cultured and characterized as described [8]. HUVEC were maintained in M199 (Sigma) supplemented with 20% FCS (Biochrome), 100 U ml<sup>-1</sup> penicillin, 100 U ml<sup>-1</sup> streptomycin, 0.25 µg ml<sup>-1</sup> fungizone and 2 mm l-glutamine (all Cambrex) at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub>:95% air. HUVEC used were between passage 3 and 8. For the proliferation assay, HUVEC were seeded in 96 well plates and incubated with medium supplemented with 5% FCS. SiNiTang was added in given concentrations. VEGF (100ng/ml, R&D Systems) served as a positive control. Cell proliferation was measured using the EZ4U kit (Biomedical) according to the manufacturer's instruction.

Tube formation was assayed as described previously [9]. Briefly HUVEC cells were plated on pre-solidified matrigel (BD) and cultured with culture medium as mentioned above for 48 hours. Dishes were imaged using a phase contrast microscope.

### Data analysis and statistics

Data was collected using a standard spread-sheet program (Excel for Mac, Microsoft). The statistical framework R (R 2.10.1) was used for statistical analysis. Analysis of variance was performed to compare multiple groups; students t-test was performed to compare two groups. If necessary bonferoni-holm correction was done to compensate for multiple testing. A p<0.05 was considered statistically significant. Data is shown as Mean±SEM

### Results

Post operative mortality was 30% in the first 48h post-MI due to stress from the operation and acute heart failure due to the creation of MI. No animals died after this period. One week post-MI animals were randomized into receiving either placebo or SiNiTang via drinking water. Liquid consumption was comparable in both groups. HPLC analysis of the prepared batches ensured constant quality (supplemental Figure 1).

Echocardiography was performed 6 weeks post-MI. Heart Rate between groups was comparable with 254±5 bpm. MI animals showed increased end diastolic diameters (Figure 1A) and reduced left ventricular fractional shortening (Figure 1B). SiNiTang (MI+T) significantly reduced left ventricular end diastolic diameters (p=0.012 MI vs. MI+T) (Figure 1A) and significantly improved left ventricular fractional shortening (p=0.037 MI vs. MI+T) (Figure 1B) compared with placebo treated MI animals.

Post-mortem analysis revealed development of significant hypertrophy in the MI group as measured by the heart weight to bodyweight ratio. SiNiTang treatment did not change the hypertrophic response post-MI (Figure 1C). Interestingly, myocardial cross sectional area was significantly reduced in SiNiTang treated animals compared to placebo (247±18µm vs 197±7µm in placebo and SiNiTang respectively p=0.026).

Exploratory examination of HE stained histology slides indicated increased vascularization in infarct tissues of SiNiTang treated animals (Figure 2A,B). Therefore immunohistological staining of von Willebrand Factor (vWF) was performed. vWF staining confirmed the presence of multiple larger vessels in SiNiTang treated animals whereas only few smaller vessels were found in placebo treated animals (Figure 2C,D).

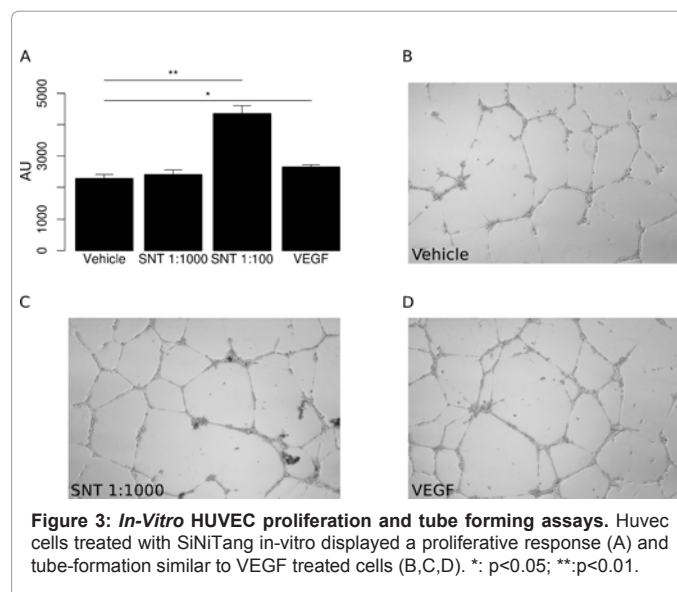
To examine effects of SiNiTang on vasculogenesis a HUVEC proliferation assay was performed. SiNiTang increased HUVEC proliferation dependent on dose. In a dilution of 1:100 SiNiTang

significantly increased vascular proliferation ( $p < 0.001$  versus vehicle) (Figure 3A). To establish whether not only proliferation but also vessel formation was affected tube formation assays were performed. Therefore HUVECs were cultured on top of matrigel mixed with vehicle, VEGF or SiNiTang (1:100) respectively. Both VEGF and SiNiTang increased tube formation after 24 hours. (Figure 3B-D)

## Discussion

In contrary to western medicine, therapy in TCM is not chosen dependent on a single symptom or disease. Diseases are viewed as caused by an imbalance within the patient's body, which then causes a characteristic symptom. Instead of directly treating the symptoms, the underlying imbalance is addressed. Thus, it differs from western evidence based medicine, where each symptom is treated based on underlying molecular mechanisms. Chinese herbal remedies contain a number of ingredients which by themselves do further contain numerous bioactive components. The interaction between these components builds a complex foundation for the effect of the remedy [10].

The here used remedy SiNiTang consists of 3 components: Radix Lateralis Aconiti Carmichaeli, Rhizoma Zingiberis Officinalis, Radix Glycyrrhizae Uralensis. The main usage of the remedy is to treat a



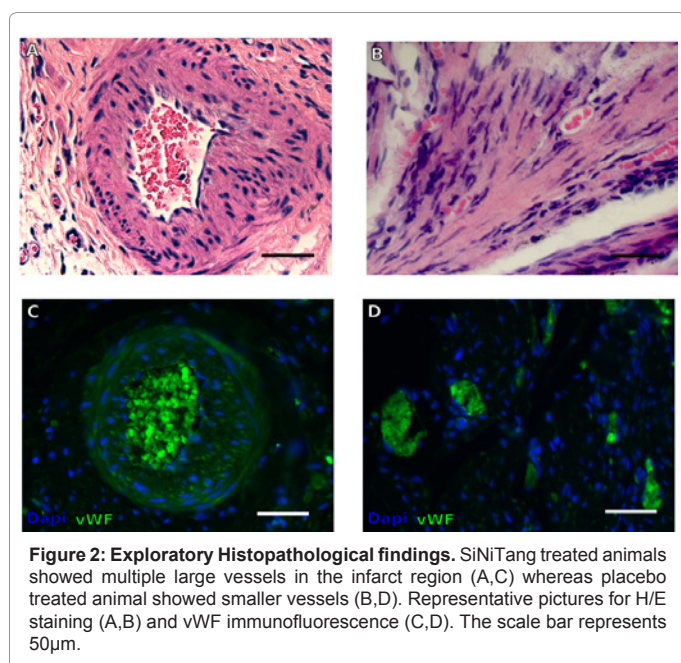
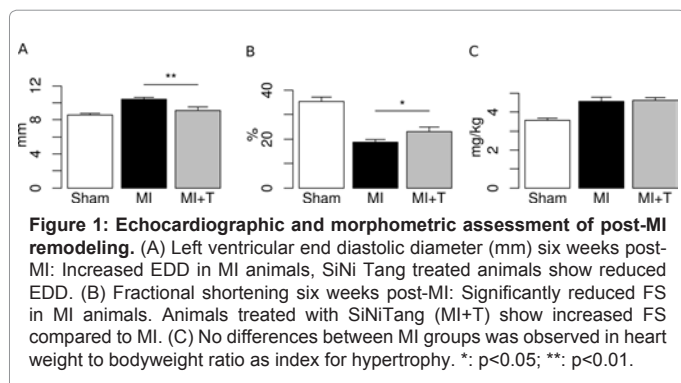
symptom titled “yang depletion”. It is characterized by cold extremities, weak pulses and lethargy. This corresponds to western diseases of myocardial infarction, heart failure and shock [5]. The remedy is mostly used for acute treatment. Nevertheless, it can be used for chronic treatment if Cinnamonum Cassia is added to balance the effects of the other ingredients [6]. Despite its importance in Chinese medicine, the remedy is researched poorly in the corresponding conditions defined in western medicine. It was examined in patients with congestive heart failure in NYHA classifications II and III, short term administration of SiNiTang in this population increased fractional shortening and decreased vascular resistance [11]. Long term treatment with SiNiTang resulted in improved fractional shortening and NYHA classification [11]. The only experimental study conducted examined the effects of SiNiTang on a rat model of endotoxic shock. Treatment with SiNiTang helped to maintain mean arterial pressure; however it did not significantly change vascular diameters or reaction [12].

We could show in our study that treatment with SiNiTang has a beneficial effect in post-MI remodeling: Administration of the drug resulted in attenuated left ventricular dilation - as indicated by end-diastolic diameters - and improved left ventricular function, as indicated by a significantly improved EF in the treatment group.

Looking for a possible method of action, multiple large vessels were found in the border-infarct and infarct area of SiNiTang treated animals, indicating neoangiogenesis. To test whether SiNiTang has a pro-angiogenic effect, HUVEC proliferation and tube formation assays were performed. SiNiTang significantly increased HUVEC proliferation and tube formation. Thus neoangiogenesis is one of the potential mechanisms by which SiNiTang could mediate its beneficial effects in post-MI remodeling and ischemic heart failure.

Other components frequently used in Chinese herbal remedies have been examined in the setting of post-MI remodeling. Of these both Ginsenoside-RG1 and puerarin, the main active component of Radix Pruarie were shown to increase angiogenesis in a similar model [13,14]. Both components were linked to an increase in VEGF.

Examining TCM in models of western medicine is challenging. The systems do not only differ in therapeutics used but also in fundamental concepts of physiology and pathology. While effects of traditional





Chinese remedies in traditional Chinese physiology and pathology are well documented. Translating from traditional Chinese pathology into western medicine is not straightforward. This study is merely limited to test whether a specific Chinese remedy can be applied to a well defined experimental pathology. Since the tested remedy is composed of four different ingredients, no statement can be made about the bioactive components responsible for the effects.

If the extensive body of Chinese remedies is used to find bioactive components interesting in western medicine, thorough understanding of Traditional Chinese medicine is needed. Further studies are needed to be able to translate pathologies between the two systems.

Well established experimental models of diseases can be used to evaluate Chinese herbal remedies either in their composite form or with isolated active components. SiNiTang attenuates post-MI ventricular dilation and preserves ventricular function. Further it increases new vessel formation both in-vivo and in-vitro. Additional studies are needed to elucidate underlying molecular mechanisms.

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