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Protective Effect of Wenxin Granula on Heart from Myocardial Infarction through Regulating Intracellular Ca^{2+}

LI Xiao-xue¹, LI Xue-lian¹, CHU Wen-feng^{1, 2}, CAI Rui-jun¹, SHI Yong-fang¹, XU Chao-qian^{1, 2}, SHAN Hong-li^{1, 2}, WANG Xing-yang¹, LU Yan-jie^{1, 2}, YANG Bao-feng^{1, 2*}

1. Department of Pharmacology, Harbin Medical University, Harbin 150081, China

2. The State-Province Key Laboratories of Biomedicine-Pharmaceutics of China, Key Laboratory of Cardiovascular Research, Ministry of Education, Harbin Medical University, Harbin 150081, China

Abstract: **Objective** To assess the anti-arrhythmic activity and cardioprotective effects of Wenxin Granula, a traditional Chinese formula (consisting of *Salviae Miltiorrhizae Radix*, *Polygonati Rhizoma*, *Notoginseng Radix et Rhizoma*, *Nardostachyos Radix et Rhizoma*, *Angelicae Sinensis Radix*, and *Succinum*), on heart in ischemic-induced myocardial infarction (MI) rats and compare with those of Amiodarone which have been demonstrated in clinic. **Methods** Rats were randomly divided into Sham-operated (control), MI + Amiodarone [5 mg/(kg·d)] (MI), and MI + Wenxin Granula [10 mg/(kg·d)] groups and left anterior descending coronary artery was occluded in each group. After left anterior descending for 12 h, standard lead II of administration electrocardiogram was recorded in order to analyze the occurrence of arrhythmia. After one month, the size of the infarct area of heart was evaluated by TTC staining method and haemodynamic function was assessed to detect the heart function. Laser scanning confocal microscope and the technique of patch clamp were used to detect the intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) and L-type calcium current ($I_{\text{Ca-L}}$), respectively. **Results** Both Wenxin Granula [10 mg/(kg·d)] and Amiodarone [5 mg/(kg·d)] could markedly decrease the incidence of arrhythmia in heart of rats which were subjected to ischemic injury. After one month, Wenxin Granula could significantly decrease mortality to 22.22% and reduce the infarct area ($P < 0.05$), but Amiodarone did not. The mechanism may involve that Wenxin Granula attenuated $[\text{Ca}^{2+}]_i$ decreasing in MI rats. Additionally, Wenxin Granula could obviously ameliorate the impaired heart function of MI rats by decreasing the elevated left ventricular end-diastolic pressure and increasing the attenuated maximum change velocity of left ventricular pressure in the isovolumic contraction or relaxation period. On the other hand, electrophysiological experiment results revealed that Wenxin Granula administration one month later also increased the reduced $I_{\text{Ca-L}}$ density in rat ventricular myocytes in MI rats. The results of LSCM showed that Wenxin Granula could recover the amplitude of $[\text{Ca}^{2+}]_i$ decreased by heart failure during long term. **Conclusion** Wenxin Granula could not only inhibit the incidence of arrhythmia but also decrease the mortality, which was accompanied by recovering the amplitude of $[\text{Ca}^{2+}]_i$. This protective effect of Wenxin Granula may partially be mediated through changing $I_{\text{Ca-L}}$ as well as increasing $[\text{Ca}^{2+}]_i$.

Key words: heart failure; intracellular Ca^{2+} ; L-type calcium current; myocardial infarction; Wenxin Granula

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Introduction

Ischemic heart disease is a leading cause of death in most of the developing countries as seen over past

quarter-century and the associated financial burden is increasing every year (Rosamond *et al*, 2008; Gaziano, 2005). Myocardial infarction (MI) is a common disorder

* Corresponding author: Yang BF, an academician of Chinese Academy of Engineering Address: Harbin Medical University, 194 Xuefu Road, Nangang District, Harbin 150081, China Tel/ Fax: +86-451-8667 1354 E-mail: yangbf@ems.hrbmu.edu.cn; shanhongli@yahoo.com.cn Received: February 18, 2011; Revised: March 19, 2011; Accepted: March 24, 2011

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in clinic, and it is characterized by a loss of excitability followed by cascades of ionic, functional, and metabolic abnormalities, which can induce arrhythmia and sudden cardiac death (Waldo *et al*, 1996). Especially in the long-term, MI-induced abnormal heart function is a common syndrome in patients with ischemic heart diseases and is becoming a major cause of mortality (Colucci, 1997; Gomez *et al*, 2001). So reducing the mortality and preventing of MI are of utmost importance.

Cardiovascular protective drug therapies have specific clinical indications. Although many clinic trails have confirmed that some western medicinal drugs with protective effects on heart could reduce the incidence of arrhythmia, they failed to prevent cardiac death triggered by chronic ischemic heart failure which often caused “sufficiently high risk” for death events in patients with MI during long-term therapy. Traditional Chinese medicine formulas have prominent advantages because of their stable curative effects with low toxicity, which have been used to treat heart disease by aiming at arousing the body’s potential recovery (Chen *et al*, 2010; Liang *et al*, 2010). Most importantly, some of them can effectively reduce the risk of recurrent disease and increase life expectancy (Waldo *et al*, 1996). Wenxin Granula, a Chinese medicinal herb formula composed of *Salviae Miltiorrhizae Radix*, *Polygonati Rhizoma*, *Notoginseng Radix et Rhizoma*, *Nardostachyos Radix et Rhizoma*, *Angelicae Sinensis Radix*, and *Succinum*, can elevate coronary blood flow, degrade myocardial consumption of oxygen, inhibit platelet aggregation, improve heart muscle ischemia, and cure arrhythmia (Doroshov, 1983). These herbs used together would synergize the desirable effects on the treatment of heart disease. There were a mass of clinical researches which have provided enough evidences to the efficacy and safety of the multiple components in Wenxin Granula protecting against arrhythmia and preventing heart failure. Meanwhile, it was also reported that Wenxin Granula had positive clinical curative effect and was safe to patients with silent myocardial ischemia (SMI) accompanied by premature ventricular contraction (PVC) after MI during long term, and clinical application value for the treatment of coronary artery disease (Sun *et al*, 2002; Zhou, Hu, and Mu, 2007; Brutsaert, 2003). However, there is no evidence confirming about the mechanism

of Wenxin Granula protecting against the arrhythmia of postischemia even reducing the mortality of patients with heart failure.

However, cardiac function, in particular, the cardiac electrical activity, is a well-organized control by an array of ion channels that activate in a manner with a delicate balance between inward and outward ion currents (Yang *et al*, 1998). On the other hand, intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) overloading or serious absence is responsible for the heart function and cell abnormality which are associated with ischemia and reperfusion injury. $[\text{Ca}^{2+}]_i$ overloading contributes to irreversible cell damage and ischemia is associated with impaired calcium homeostasis (Brutsaert, 2003). Short-term ischemic tissue injury causes an acute increase in free $[\text{Ca}^{2+}]_i$ that leads to diminished recovery of heart and then results in the heart failure in the long term. However, heart failure is a progressive disease, and in many cases, myocardial function will eventually deteriorate in the face of unchanged or reduced haemodynamic load (Nattel *et al*, 2007). Fascination with the role of Ca^{2+} has proliferated into all aspects of our understanding of normal cardiac function and the progression of heart disease, including induction of arrhythmia, heart failure, and sudden death (Brutsaert, 2003; Bodi *et al*, 2005). The cardioprotective effects of drugs in MI patients were the results of their anti-injury actions and played an important role in the altered Ca^{2+} signaling observed in this model of heart failure. The $[\text{Ca}^{2+}]_i$ serious absence is the hallmark of the mortality induced by ischemic heart disease (Brutsaert, 2003; Camerino, Tricarico, and Desaphy, 2007; Chu *et al*, 2006). The aim of the present study is to investigate the underlying electrophysiological mechanisms of anti-arrhythmic action in a rat model of MI by Wenxin Granula and the possible involvement of $[\text{Ca}^{2+}]_i$ regulation.

Materials and methods

Drugs

Wenxin Granula for ig administration was produced by Pharmaceutical Co., Ltd., Shandong Buchang, and was directly purchased from Beijing Tongrentang Pharmacy, mainly consisted of medicinal components, including *Salviae Miltiorrhizae Radix*,

Polygonati Rhizoma, *Notoginseng Radix et Rhizoma*, *Nardostachyos Radix et Rhizoma*, *Angelicae Sinensis Radix*, and *Succinum*. The quality standards were strictly developed according to the 2010 edition of *Chinese Pharmacopoeia* Appendix VI B. Amiodarone (Sigma-Aldrich) was used here as a positive control drug.

Animals

Male Wistar rats (the Animal Center of the 2nd Affiliated Hospital of Harbin Medical University, China) were used in this study. Rats (weighing 200–300 g) were conditioned for one month at $(23 \pm 1)^\circ\text{C}$ with a constant humidity of $(55 \pm 5)\%$, under a circle of 12 h dark-light. At the same time rats had free access to food and tap water according to GLP regulations of the Ethic Committees of Harbin Medical University and confirmed with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996).

Rat model of MI

Rats were randomly divided into Sham-operated (control), MI + Amiodarone [5 mg/(kg·d)] (MI), and MI + Wenxin Granula [10 mg/(kg·d)] groups. MI model was established as described previously (Zhang *et al*, 2006; Yang *et al*, 2005; Loot *et al*, 2004; Alvarez *et al*, 2004). Before operation, rats were pre-administrated with Wenxin Granula and Amiodarone for 7 d, respectively. Until the day 8, rats were initially anesthetized with Pentobarbital (40 mg/kg, iv) and placed in the supine position with the upper limbs taped to the table. A 1–1.5 cm incision was made along the left side of the sternum. The thorax was cut open at the point of the most pronounced cardiac pulsation and the right side of the chest was pressed to push the heart out of the thoracic cavity. The left anterior descending coronary artery (LADCA) was occluded and then the chest was closed. All surgical procedures were performed under sterile conditions. Then the MI models were divided into 12 h-group and one month-group respectively. During the next 30 d, drugs were consecutively administered to one month-group every morning. Until the day 31, the heart was removed for Langendorff perfusion or the tissues within the ischemic, border, and non-ischemic zone distal to the ischemic zone were dissected for measurement. However, in the 12 h-group, the heart was

removed immediately on the day 9 for perfusion and then to detection.

Measurements of infarct size

Ventricular tissues were dissected and kept overnight at -4°C . Frozen ventricles were sliced into 2 mm thick sections, and then incubated in 1% triphenyltetrazolium chloride at 37°C in 0.2 mol/L Tris buffer (pH 7.4) for 30 min. While the normal myocardium was stained brick red, the infarcted areas remained unstained. Size of the infarct area was estimated by the volume and weight as a percentage of the left ventricle (Yang *et al*, 2005).

Measurement of arrhythmia

The standard limb lead II ECG was recorded when the rats were anesthetized for 1 h before and 11 h after coronary artery ligation. After one month, the long-term groups were also repeated the same process as described above. The incidence of arrhythmia was registered and evaluated in accordance with the Lambeth Conventions (Walker *et al*, 1988).

Measurement of haemodynamic function

Haemodynamic function was assessed before and one month after infarction by determining left ventricular end-diastolic pressure (LVEDP), and time derivatives of pressure were measured during contraction ($+dp/dt_{\max}$) and relaxation ($-dp/dt_{\max}$) recorded on a polygraph.

Myocyte isolation and whole-cell patch-clamp techniques

The myocytes dissociation procedure was similar to that described previously (Yang *et al*, 2007). The cardiomyocytes were transferred to a chamber mounted on an inverted microscope (Nikon Diaphot, Nikon) for electrophysiological recording in Tyrode's solution. Whole-cell patch-clamp recording techniques have been described in detail elsewhere (Zhang *et al*, 2006). Ionic currents were recorded in the whole-cell voltage-clamp mode with an Axo-patch 200B amplifier (Axon Instruments). Borosilicate glass electrodes had tip resistance of 1–3 M Ω when filled with pipette solution (KCl 130 mmol/L, MgCl₂ 1 mmol/L, Hepes 10 mmol/L, EGTA 10 mmol/L, and Mg-ATP 5 mmol/L; pH 7.25 with KOH). Junction potential was zeroed before formation of the membrane-pipette seal in Tyrode's solution and was not corrected for our data analyses. Experiments were conducted at $(36 \pm 1)^\circ\text{C}$.

Series resistance and capacitance were compensated and leak currents were subtracted.

[Ca²⁺]_i measurement

Cytosolic free Ca²⁺ was measured by confocal microscopy and flow meter analysis (Chossat *et al*, 2001). After the single cardiomyocyte isolated from rats ventricular was adhered to the cover-slips of the chamber, cells were rinsed once with the standard Tyrode's solution (NaCl 126 mmol/L, KCl 5.4 mmol/L, HEPES 10 mmol/L, NaH₂PO₄·2H₂O 0.33 mmol/L, MgCl₂·6H₂O 1.0 mmol/L, CaCl₂ 1.8 mmol/L, and Glucose 10 mmol/L; pH value was adjusted to 7.40 ± 0.05 with NaOH) and then incubated with a working solution containing Fluo-3/AM (20 mmol/L Molecular Probes, Eugene, OR, USA) and Pluronic F-127 (0.03%) at 37 °C for 45 min. After loading, cells were washed with the standard Tyrode's solution to remove the extracellular Fluo-3/AM. The images were captured by a confocal microscope (488 nm excitation, 530 nm emission). KCl (60 mmol/L) was added between the 2nd and 3rd scan with 10 s interval scanning course for 30 times and the images were stored in hard disks. Fluorescent intensities before (FI₀) and after (FI) the KCl administration were recorded. Fold change in [Ca²⁺]_i was represented by the ratio of FI/FI₀.

Data analysis

Group data are expressed as $\bar{X} \pm s$ and analyzed by GraphPad Prism 5.0 and Sigmaplot 9.0 software. One-way ANOVA followed by Bonferroni's post-hoc test was used to compare group data from animal experiments. Chi-square analysis was used to compare incidence of arrhythmia in different groups. Kruskal-Wallis analysis was used to compare arrhythmia scores. A two-tailed $P < 0.05$ was taken to indicate a statistically significant difference.

Results

Wenxin Granula protects against ischaemic-induced heart injury

After left anterior descending (LAD) for 12 h, standard lead II ECG was recorded in order to analyze the occurrence of arrhythmia. No spontaneous arrhythmia was observed in control group. In MI group, ECG presented as an obvious elevation of ST segment. Coronary occlusion induced ventricular tachycardia (VT) and ventricular fibrillation (VF) (Fig. 1A) and elevated the score of arrhythmia to 4.43 ± 0.54 ($n = 15$, $P < 0.05$). Administration of Wenxin Granula [10 mg/(kg·d)] can lower the score of arrhythmia to 2.94 ± 0.73 (Fig. 1B) and the duration of arrhythmia had been attenuated ($n = 15$, $P < 0.05$) (Fig. 1C). The incidence of VT was elevated to 92% in MI group and decreased

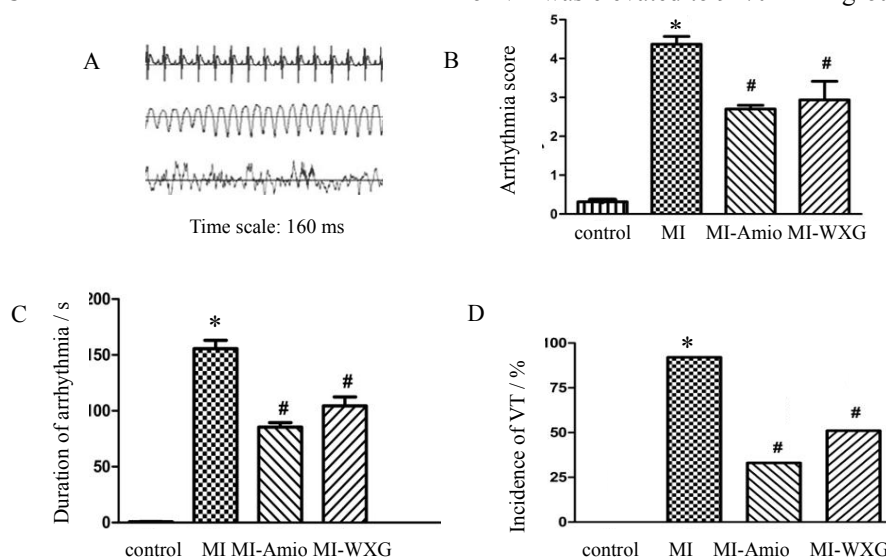


Fig. 1 Effect of Wenxin Granula on arrhythmia

A: Examples of ventricular tachycardia and ventricular fibrillation

B: Both Wenxin Granula and Amiodarone lowered the arrhythmia score during 12 h of MI

C: Compared with MI group, Wenxin Granula and Amiodarone made arrhythmia lasting shorter time as panel right shows

D: Both Wenxin Granula and Amiodarone decreased the incidence of VT in 12 h of MI. In control group, there existed nearly no arrhythmia as panel left shows (* $P < 0.05$ vs control; # $P < 0.05$ vs MI, $n = 15$)

to 51% ($n = 15$, $P < 0.05$) by administration of Wenxin Granula (Fig. 1D). No significant differences were found between the Wenxin Granula and Amiodarone-treated group.

Decrease in mortality of MI

In order to investigate the long-term effect of Wenxin Granula on the MI rats, we prepared the model group and the administration of drug group for one month (long-term), respectively. Results showed that administration of Wenxin Granula and Amiodarone could reduce the size of infarct area by approximately 30% and 10%, respectively, compared with MI group ($P < 0.05$, $n = 6$) (Fig. 2A). Furthermore, we found that MI (one month post-infarction) induced the mortality to a ratio of 42.9% ($P < 0.05$, $n = 15$). Interestingly, the mortality was decreased to 22.22% ($P < 0.05$, $n = 15$) in Wenxin Granula-treated group. However, Amiodarone seemed to have no significant effect on mortality rate of MI rats (Fig. 2B).

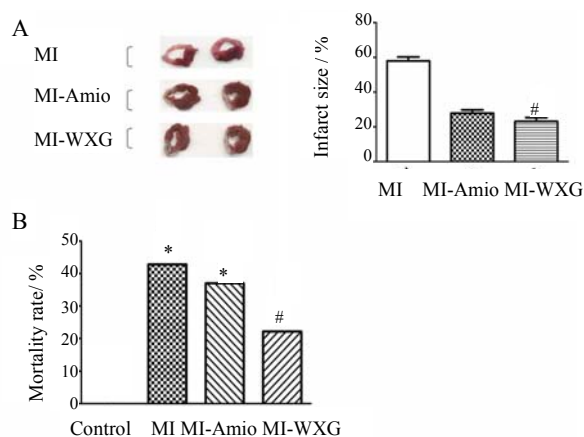


Fig. 2 Long-term effect of Wenxin Granula on MI

A: Examples of infarcted area of the left ventricular walls (coronal sections) isolated from MI rats after one month. Left panel: the infarcted area was stained white. Right panel: mean data of infarct size ($^{\#}P < 0.05$ vs MI, $n = 7$)

B: Effect of Wenxin Granula and Amiodarone on mortality rate of rats suffered from ligation of left coronary anterior descending limb in one month ($^*P < 0.05$ vs control; $^{\#}P < 0.05$ vs MI; $\bar{X} \pm s$, $n = 10$)

Table 1 shows the haemodynamic parameters when administration of Wenxin Granula after occlusion in long

term (one month) on these parameters. MI caused significant increases in LVEDP as well as reduction of $\pm dp/dt_{\max}$ indicating impaired cardiac systolic function. Strikingly, compared with Amiodarone, administration with Wenxin Granula ameliorated these deleterious changes; LVEDP was markedly reduced, and the decrease in $\pm dp/dt_{\max}$ measured in the infarcted rats were diminished. These results suggested that Wenxin Granula attenuated the symptoms of MI animals suffering from heart failure.

Effects of Wenxin Granula on L-type calcium current (I_{Ca-L}) of rat ventricular myocytes

Because long-term ischemia results in heart failure which are accompanied by reduction of I_{Ca-L} , the changes of I_{Ca-L} in rat ventricular myocytes isolated from the heart of MI rats for one month were detected. Fig. 3 shows the effects of Wenxin Granula on I_{Ca-L} in rat ventricular myocytes. When the holding potential was -40 mV and the test potential was $+10$ mV, the inward I_{Ca-L} reached its maximum level. I_{Ca-L} was significantly reduced in MI rat cardiomyocytes at test potentials ranging from -30 to $+70$ mV. At the test potential of $+10$ mV, Wenxin Granula increased I_{Ca-L} densities of MI from -6.5 ± 1.6 to -8.8 ± 1.8 pA/pF (by about 35%, $P < 0.05$). Surprisingly, Amiodarone exhibited the inhibitory effect on I_{Ca-L} densities. It indicated that MI-induced arrhythmia could trigger the decrease of I_{Ca-L} during long term, and may act as a key pathway in prevention of heart failure-induced mortality.

Wenxin Granula enhance the amplitude of $[Ca^{2+}]_i$ decreased by MI-induced heart failure

It is well known that release of Ca^{2+} into cytosolic for heart failure during the long term in many cases and overload of $[Ca^{2+}]_i$ play a key role in promoting death (Yang *et al.*, 2005). We stimulated the rat ventricular myocytes in each group with KCl (60 mmol/L) to observe the change of the elevated amplitude of the $[Ca^{2+}]_i$ by confocal microscopy. The results showed that MI triggered a sharp decline of FI/FI_0 one month later, reflecting a decrease of $[Ca^{2+}]_i$ in MI group compared

Table 1 Haemodynamic parameters after one month of treatment ($\bar{X} \pm s$, $n = 7$)

Group	HR / bpm	LVEDP / mmHg	$\pm dp/dt_{\max} / (mmHg \cdot s^{-1})$	$-dp/dt_{\max} / (mmHg \cdot s^{-1})$
Control	327 $\pm$ 25	3.4 $\pm$ 1.0	3420 $\pm$ 276	2877 $\pm$ 636
MI	348 $\pm$ 29	13.2 $\pm$ 2.3 [*]	2632 $\pm$ 326 [*]	1996 $\pm$ 318 [*]
MI + Amio	347 $\pm$ 23	12.0 $\pm$ 1.6 [*]	2416 $\pm$ 124 [*]	1822 $\pm$ 96 [*]
MI + WXG	338 $\pm$ 28	6.6 $\pm$ 1.8 [#]	3189 $\pm$ 145 [#]	2682 $\pm$ 319 [#]

One-way ANOVA followed by Bonferroni's post-hoc test. ^{*} $P < 0.05$ vs control; [#] $P < 0.05$ vs MI

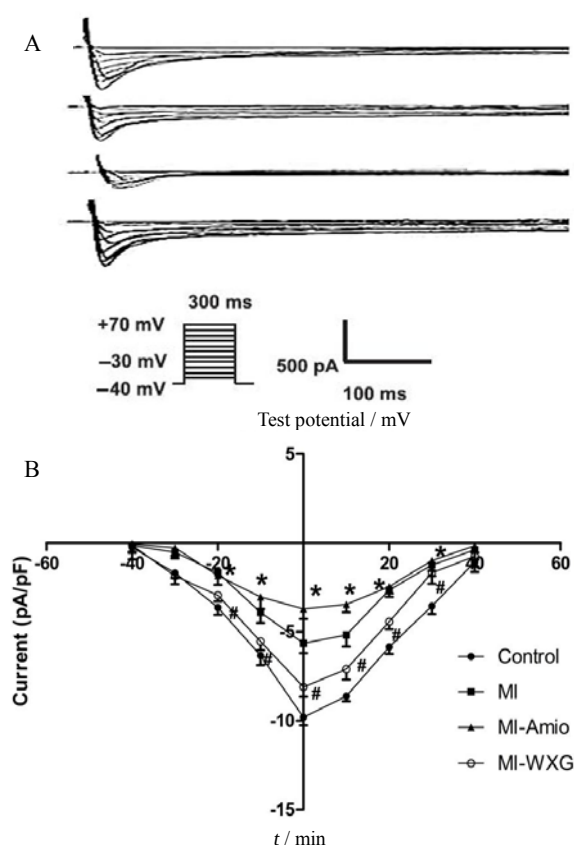


Fig. 3 I_{Ca-L} changes of rat ventricular myocytes in each group

A: I_{Ca-L} current traces activated by depolarizing voltage pulses from -30 to +70 mV

B: current-voltage relationship of the I_{Ca-L} in each group

* $P < 0.05$ vs control; # $P < 0.05$ vs MI. $n = 6$ cells from 3 rats

with the control group. But in Wenxin Granula group, the decrease of $[Ca^{2+}]_i$ has been normalized similarly to that of the control group. On the other hand, the amplitude of $[Ca^{2+}]_i$ in Amiodarone group was also observed, although Amiodarone could prevent arrhythmia in short period, it did not significantly elevate or even regulate the $[Ca^{2+}]_i$ during long term (Fig. 4A and B). These results suggested that MI-induced elevation of $[Ca^{2+}]_i$ overload resulted in heart failure which was prevented by Wenxin Granula, which might be involved in regulation of $[Ca^{2+}]_i$ through ion channels.

Discussion

There are important fundamental implications of our findings. MI results in a dramatic disorder of automaticity and conduction, neurohumoral changes, and structural remodeling, all of which contribute to the

development of electrical disturbance that leads to life threatening cardiac arrhythmia (de Bakker and van Rijen, 2007). In this study, administration of Wenxin Granula significantly diminished the incidence of arrhythmia in the first 12 h of MI, which corresponds to the peri-infarction period during which phase II ischaemic arrhythmia often occurs. Consistent with our previous study (Yang *et al*, 2007), individuals surviving from MI-presented decrease of I_{Ca-L} density and up-

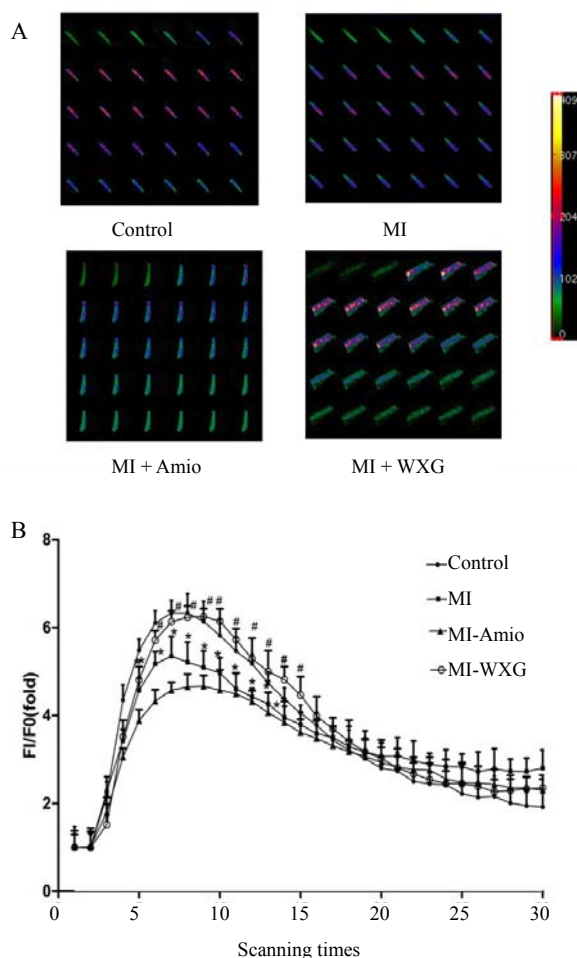


Fig. 4 Comparison of the changes of $[Ca^{2+}]_i$ in each group

A: The images of change of $[Ca^{2+}]_i$; Control group; one month after MI; Amiodarone group; Wenxin Granula could recover the decreased $[Ca^{2+}]_i$; The colorful zoom of yellow indicates the maximum of change of Ca^{2+} , red and blue indicates the decreased Ca^{2+} in the state of recovery, and green indicates the minimum of change of Ca^{2+} compared with the other three color

B: The summarize of panel A, MI happened for one month, the elevated amplitude of $[Ca^{2+}]_i$ decreased. Wenxin Granula can raise the decreased of $[Ca^{2+}]_i$ induced by MI, while Amiodarone lowers the amplitude of $[Ca^{2+}]_i$. Fluorescence intensity (FI) changes were normalized and expressed as FI/FI₀ values, where FI₀ was the fluorescence intensity at base-line and FI was the change of FI after KCl was added (* $P < 0.05$ vs control. # $P < 0.05$ vs MI. $n = 10$ cells from 3 rats)

regulation of $[Ca^{2+}]_i$. The data presented in this study are the first to reveal the protective effects of Wenxin Granula against arrhythmia in animal models and its cellular and ionic mechanisms. Cardiac function, in particular, the cardiac electrical activity, is a well-organized control by an array of ion channels that activate in a manner with a delicate balance between inward and outward ion currents. Receiving an incoming impulse, cardiac cells excite with rapid membrane depolarization followed by a relatively slow repolarization process. Repolarization disorders, either excessive slowing or accelerating of the repolarization rate, can cause cardiac electrical perturbations of arrhythmia. Anti-arrhythmic drug should also affect multiple channels, be able to restore normal sinus rhythm in all patients, and keep patients free from further episodes of arrhythmia. Taking this into consideration, the currently available drugs are far from perfection. One increasingly well-recognized risk of anti-arrhythmic therapy is the possibility of provoking new arrhythmia, with potentially life-threatening consequence. So we want to explore a drug with safety and effectiveness.

Although it was found that Wenxin Granula prevented arrhythmia and inhibited the mortality induced by MI, which had association with regulating $[Ca^{2+}]_i$, the mechanism needed further investigated. As is well known, calcium influx and efflux through the plasma membrane are normally in equilibrium and go out of balance during ischemia with Ca^{2+} influx exceeding Ca^{2+} efflux. The most important mechanism for removal of Ca^{2+} from the cell is the Na^+/Ca^{2+} exchanger, and it is responsible for 77% of the Ca^{2+} extrusion. MI was induced by coronary artery ligation, in response to which heart presented changes in resting potential, conduction velocity, $[Ca^{2+}]_i$ concentrations, and repolarization, any one of which may create arrhythmia. During ischemia, the Na^+/Ca^{2+} exchanger is less efficient because of an increase in $[Na^+]_i$ and $[H^+]_i$. An increase in $[Na^+]_i$ promotes the reversed mode of the Na^+/Ca^{2+} exchanger whereby Ca^{2+} is entering the cell and Na^+ is removed. All mechanisms responsible for an increase in $[Na^+]_i$ thus also contribute to the increase in $[Ca^{2+}]_i$ via the exchanger. The efficiency of the exchanger is furthermore reduced by the acidosis and the influence of radicals. Whereas Ca^{2+} efflux is

reduced, Ca^{2+} influx is increased during metabolic inhibition. The role of L-type Ca^{2+} channels in this respect is negligible. So the channels carrying Ca^{2+} inward become more important during ischemia as they are activated by radicals.

On the other hand, the maintenance of cardiomyocytes resting membrane potential depends on the extracellular K^+ , and the increase of K^+ causes the depolarization of membrane. KCl added into the extracellular solution resulted in the opening of voltage-dependent calcium channel. The influx of Ca^{2+} evokes the Ca^{2+} -induced Ca^{2+} release, which induces a great quantity of Ca^{2+} release from the cells, resulting in an obvious increase of $[Ca^{2+}]_i$ (Rekalov *et al*, 2003). In order to see if there were changes among control, MI, and drug-administration groups, we used KCl to stimulate the $[Ca^{2+}]_i$. The findings revealed that $[Ca^{2+}]_i$ decreased in MI group which might be caused by heart failure (Korkmaz *et al*, 2009).

This phenomenon may explain that the time course of ventricular dysfunction is arbitrarily divided into such two periods: MI produces cellular death and thus leads to complete loss of contractile function in the infarcted region (acute phase), whereas there is no necessary change in contractile function in other regions; In survivors, there is a remodeling of the noninfarcted area that underlies the second phase of dysfunction. Heart failure is frequently seen during and after this period of remodeling.

Accordingly, when heart failure happened, the function of L-type calcium channel decreased, through which the influx of calcium had been depressed (Lai *et al*, 1999). Wenxin Granula improved the cardiac function through increasing the influx of calcium, which presented the elevated amplitude of $[Ca^{2+}]_i$ induced by KCl. And as a common anti-arrhythmic drug, Amiodarone could degrade the $[Ca^{2+}]_i$ in MI rats, but far away from the normal flux, which might mean Amiodarone could not recover the cardiac function when used for long-term, on the contrary, would aggravate the heart failure. However, it was observed that Wenxin Granula had obvious anti-arrhythmic effect when long-term used.

In summary, the results demonstrated that Wenxin Granula could decrease the mortality rate of long-term MI rats, also have anti-arrhythmic effect on animal

models, whose probably underlying mechanism was involved in changing the I_{Ca-L} density and recovering the $[Ca^{2+}]_i$, and it may be safer than commonly used western medicine such as Amiodarone.

In the present study, these protective effects of Wenxin Granula in heart driven by what ingredient in the formula still needs to be identified. In the future, we will investigate the effects of individual ingredients in the formula on the heart. Thus, confirming the mechanism of the potential formula to protect against death which is induced by ischemic arrhythmia in long term is important to enhance clinical management in heart disease in the future.

Conclusion

The present study revealed several findings. First, it was demonstrated that Wenxin Granula could produce cardioprotective effects in MI rats during short time. Second, Wenxin Granula could act as a kind of traditional Chinese formula and especially exhibit the effect on decreasing mortality during long-term therapy. Third, Wenxin Granula produced protective effects against death induced by ischaemic arrhythmia, possibly partially by decreasing the elevation of $[Ca^{2+}]_i$ induced by MI during long term via inhibiting I_{Ca-L} density. The data in this study suggested that Wenxin Granula could inhibit the prolongation of the action potential and recover the influx of Ca^{2+} . These findings not only help us understand the mechanisms underlying long-term ischaemic cardioprotective effects of Wenxin Granula but also conceptually advance our view of traditional Chinese formula that may serve as potential therapeutic drug in clinic.

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Tianjin Branch

Tianjin Institute of Pharmaceutical Research
308[#] An-shan West Road, Nankai District, Tianjin, China
Post Code: 300193
E-mail: chm@tiprpress.com
Tel: +86-22-2747 4913; 2300 6821
Fax: +86-22-2300 6821
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Institute of Medicinal Plant Development
Malianwa North Road, Haidian District, Beijing, China
Post Code: 100193
E-mail: bjchm@tiprpress.com
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