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RESEARCH PAPER

Tanshinone IIA protects against sudden cardiac death induced by lethal arrhythmias via repression of microRNA-1

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Background and purpose: Tanshinone IIA is an active component of a traditional Chinese medicine based on *Salvia miltiorrhiza*, which reduces sudden cardiac death by suppressing ischaemic arrhythmias. However, the mechanisms underlying the anti-arrhythmic effects remain unclear.
Experimental approach: A model of myocardial infarction (MI) in rats by ligating the left anterior descending coronary artery was used. Tanshinone IIA or quinidine was given daily, before (7 days) and after (3 months) MI; cardiac electrical activity was monitored by ECG recording. Whole-cell patch-clamp techniques were used to measure the inward rectifying K^+ current (I_{K1}) in rat isolated ventricular myocytes. Kir2.1 and serum response factor (SRF) levels were analysed by Western blot and microRNA-1 (miR-1) level was determined by real-time RT-PCR.
Key results: Tanshinone IIA decreased the incidence of arrhythmias induced by acute cardiac ischaemia and mortality in rats 3 months after MI. Tanshinone IIA restored the diminished I_{K1} current density and Kir2.1 protein after MI in rat ventricular myocytes, while quinidine further inhibited I_{K1}/Kir2.1. MiR-1 was up-regulated in MI, possibly due to the concomitant increase in SRF, a transcriptional activator of the miR-1 gene, accounting for decreased Kir2.1. Treatment with tanshinone IIA prevented increased SRF and hence increased miR-1 post-MI, whereas quinidine did not.
Conclusions and implications: Down-regulation of miR-1 and consequent recovery of Kir2.1 may account partially for the efficacy of tanshinone IIA in suppressing ischaemic arrhythmias and cardiac mortality. These finding support the proposal that *miR-1* could be a potential therapeutic target for the prevention of ischaemic arrhythmias.
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Keywords: Tanshinone IIA; ischaemic arrhythmia; sudden cardiac death; microRNA-1; I_{K1}; Kir2.1; SRF

Abbreviations: IHD, ischaemic heart disease; I_{K1}, inward rectifying K^+ current; MI, myocardial infarction; SCD, sudden cardiac death; SRF, serum response factor

Introduction

Sudden cardiac death (SCD) is one of the major causes of natural death, causing approximately one million of adult deaths in China each year. About 75% of the SCD cases are linked to a previous myocardial infarction (MI), and 80% are linked to coronary artery disease. Increased risk of arrhythmias accompanies all types of heart disease, especially with MI. Unfortunately, conventional anti-arrhythmic drugs, the ion channel antagonists, such as the class IA anti-arrhythmic compound, quinidine (Walker, 2006), have been proven ineffective in healing the electrical remodelling process or reducing SCD (Clements-Jewery *et al.*, 2005). Therefore, a major goal in cardiovascular medicine is to develop rational approaches to prevent arrhythmias and the consequent SCD.

Tanshinone IIA is a monomer of phenanthrenequinones extracted from the root of *Salvia miltiorrhiza* (Figure 1). An extract of *S. miltiorrhiza*, also known as Danshen, has been used to prevent and cure ischaemic heart disease (IHD) for thousands of years in China (Cheng, 2007). Tanshinone IIA, the key active ingredient of extracts of *S. miltiorrhiza* for treatment of IHD, has been shown to exhibit a variety of biological activities including cardiovascular action, antioxidative,

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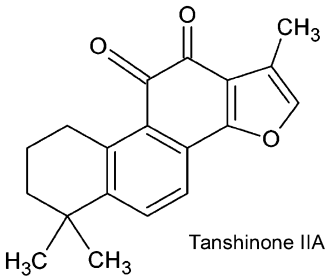

Figure 1 The chemical structure of tanshinone IIA.

anticoagulant, anti-atherosclerosis, anti-apoptosis and anti-hypertrophic actions (Adams *et al.*, 2006; Cheng, 2007; Yang *et al.*, 2007b; Gao *et al.*, 2008; Yang *et al.*, 2008). The cardioprotective activities of tanshinone IIA, such as reduction of myocardial infarct size, decrease of myocardial consumption of oxygen, have been confirmed in patients and well recognized in clinical practice. Studies demonstrate approximately 86% patients with IHD were relieved by treatment with tanshinone IIA (Cai *et al.*, 2008). However, the cardioprotective mechanisms of tanshinone IIA have not been elucidated.

Since the discovery of microRNA (miRNA), a type of single-stranded and non-coding RNAs of approximately 22 nucleotides long, more than 700 miRNAs have been identified in humans (Divakaran and Mann, 2008). MicroRNAs can regulate gene expression by binding to the 3′-untranslated regions (UTRs) of mRNAs through inexact sequence matching, targeting numerous mRNAs for translational inhibition or degradation, and may regulate up to 20–30% of all genes (Xie *et al.*, 2005). MicroRNA-1 (miR-1) is preferentially expressed in adult cardiac and skeletal muscle tissues, manifesting distinct functions (Zhao *et al.*, 2005; Chen *et al.*, 2006; Latronico *et al.*, 2007; McCarthy and Esser, 2007; Zhao *et al.*, 2007). Recent studies show that miR-1 plays important roles in regulating myogenesis (Rao *et al.*, 2006), contributing to myogenic growth and differentiation (Kloosterman and Plasterk, 2006; Zhao *et al.*, 2007). MiR-1 is also involved in pathophysiological processes relevant to human cardiac disease, such as development of cardiac hypertrophy (Sayed *et al.*, 2007), coronary artery disease, ischaemic arrhythmias (Yang *et al.*, 2007a) and cardiomyocyte apoptosis (Xu *et al.*, 2007). Overexpressed miR-1 is arrhythmogenic in ischaemic and normal hearts (Yang *et al.*, 2007a).

In light of the established pharmacological effects of tanshinone IIA and the deleterious role of miR-1 in IHD, we proposed that tanshinone IIA might produce its beneficial actions by affecting miR-1 expression. Our observations supported this proposal as we found that: tanshinone IIA was involved in the regulation of miR-1 that is important for electrical remodelling and arrhythmias in MI rats. This is probably one of the mechanisms by which tanshinone IIA attenuates MI-induced arrhythmias and the longer-term mortality due to SCD.

Methods

Rat model of myocardial infarction

All animal care and experimental procedures were in accordance with the regulations of the ethics committee of Harbin Medical University. Male Wistar rats (160 in total; 220–260 g) were randomly divided into four groups: control, MI, tanshinone IIA-MI (Tan), quinidine-MI (Qui) group respectively. The rats were housed in a climate-controlled environment at an ambient temperature of 20°C with a 12/12 h light/dark cycle. Seven days before surgery, rats of the Tan and Qui group were pretreated with tanshinone IIA (10 $mg \cdot kg^{-1} \cdot d^{-1}$) and quinidine (10 $mg \cdot kg^{-1} \cdot d^{-1}$) respectively. The animals were anaesthetized with sodium pentobarbital (40 $mg \cdot kg^{-1}$, i.p.). A left thoracotomy was performed, and the left anterior descending coronary artery was ligated, as described previously (Yang *et al.*, 2005). After surgery, the rats were treated with the same doses of drugs for 3 months. On the 90th day, the animals were anaesthetized, and the hearts were removed for Langendorff perfusion, or the tissues were extracted for measurement of miR-1 and Kir2.1 protein levels. In addition, 6 h after surgery, the animals of the short-term group were killed for measurement of miR-1 and Kir2.1 protein levels. Mortality was calculated 3 months after MI (long-term). The Control group of rats without drugs was handled in the same manner except the coronary artery was not ligated.

Scoring of arrhythmia

Standard lead II electrocardiograms were recorded for a continuous period of 6 h after surgery. Arrhythmia scoring was as described by Curtis and Walker (1988) as follows: 0 = no arrhythmia; 1 ≤ 10 s premature ventricular contraction (PVC) and/or ventricular tachycardia (VT); 2 = 11–30 s PVC and/or VT; 3 = 31–90 s PVC and/or VT; 4 = 91–180 s PVC and/or VT, or reversible ventricular fibrillation (VF) of <10 s; 5 ≥ 180 s PVC and/or VT, >10 s reversible VF, 6 = irreversible VF.

Isolation of ventricular myocytes

Single ventricular myocytes were carefully isolated using standard enzymatic digestion techniques from the rat hearts of different groups, as described previously (Yang *et al.*, 1998; Dong *et al.*, 2004; Zhang *et al.*, 2006b). In brief, rats were anaesthetized with sodium pentobarbital, and the hearts were removed rapidly and perfused via the aorta, according to the Langendorff method. Hearts were perfused at a constant pressure (60 mmHg) with Ca^{2+}-containing Tyrode solution containing (in mM): NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, HEPES 10, $NaHCO_3$ 11.9, NaH_2PO_4 0.33, glucose 10 (pH 7.4), then switched to Ca^{2+}-free solution, followed by perfusion with the same Ca^{2+}-free solution containing 0.05% collagenase and 0.1% bovine serum albumin. All solutions were bubbled with 100% O_2 and warmed at 37°C during the perfusion procedure. Ventricular tissues of peri-ischaemic zone were separated, minced, triturated into small pieces, and stored in Kraftbruhe solution, containing (in mM): glutamic acid 70, taurine 15, KCl 30, KH_2PO_4 10, HEPES 10, $MgCl_2$ 0.5, glucose 10 and EGTA 0.5; adjusted to pH 7.4 with KOH. The cell suspension was filtered, centrifuged (440× *g* for 30 s) and resuspended in Kraftbruhe solution. Only rod-shaped Ca^{2+}-tolerant striated myocytes were studied.

Patch-clamp recording of I_{K1} (inward rectifying K^+ current) in myocytes

Whole-cell patch-clamp recordings were done using an Axopatch 200B amplifier and pCLAMP8.0 software (Axon

Instruments Inc., Union City, CA, USA), as described before (Yang *et al*., 1998; Zhang *et al*., 2006b). Standard patch-clamp electrodes were made from thin-wall glass capillaries using a horizontal puller (Narishige, Tokyo, Japan) and were heat-polished. Borosilicate glass electrodes had a tip resistance of 2–4 MΩ when filled with pipette solution. The pipette solution contained (in mM): K aspartate 140, $MgCl_2$ 5, K_2ATP 5, EDTA 5, HEPES 5 (pH 7.2). I_{K1} was measured as a Ba^{2+}-sensitive current using 300 ms steps from –120 to 50 mV (10 mV increments) from the holding potential in the presence and absence of 0.3 mM $BaCl_2$. Data were analysed with pCLAMP software and plotted as current–voltage curves. Experiments were conducted at room temperature (22–23°C).

Western blot analysis

The protein samples were extracted from rat peri-ischaemic ventricle and were rinsed in phosphate-buffered saline buffer (pH 7.4) containing protease inhibitors on ice. The tissue was homogenized and centrifuged at 4°C for 15 min at 160× *g* to remove debris and nuclei in a 10 mM Tris buffer containing 0.5 M sucrose and protease inhibitors. The supernatant was ultracentrifuged at 45 000× *g* for 30 min and resuspended with 100 µL 0.4% SDS Ripa Buffer. The protein concentrations were determined with BCA Protein Assay Kit by spectrophotometer. Protein samples were fractionated by SDS-PAGE (10% polyacrylamide gels) then transferred to PVDF membrane (Yang *et al*., 2005; Yue *et al*., 2006). The molecular basis of native I_{K1} current is mainly formed by Kir2.1 channels (current and channel nomenclature follows Alexander *et al*., 2008), so we measured the expression of Kir2.1 in MI rats. The primary antibodies, anti-Kir2.1 (Alomone Labs, Israel) and anti-SRF (serum response factor) (Santa Cruz, USA) antibodies were used, with GAPDH as an internal control.

Quantification of miR-1 and Kir2.1 mRNA levels

For quantification of KCNJ2 transcripts, conventional real-time RT-PCR was carried out with total RNA samples extracted from rat heart after MI. TaqMan quantitative assay was performed with the expression level of GAPDH as an internal control. Primers for Kir2.1 mRNA were 5′-CCGCTACAGC ATCGTCTC-3′ (forward) and 5′-TGCCCGTCTTTCTTCACA-3′ (reverse). The total RNA samples were isolated with *mir*Vana™ miRNA Isolation Kit (Ambion, USA) from rat hearts post-MI. MiR-1 levels were measured using the *mir*Vana™ qRT-PCR miRNA Detection Kit (Ambion, USA), which is a type of quantitative reverse transcription-PCR kit and was used in conjunction with real-time PCR with SYBR Green I for quantification of miR-1 transcripts (Luo *et al*., 2007). Primers for real-time PCR were 5′-GGGGTGGAATGTAAAGAA-3′ (R-*miR-1*-forward) and 5′-TGCGTGTCGTGGAGTC-3′ (R-*miR-1*-reverse). Variations in expression of miR-1 between different RNA samples were calculated after normalization to U6. Genbank accession numbers rattus norvegicus mature miR-1: DQ066650. The sequence is complementarity between rat miR-1 and its target sites in the 3′-UTRs of KCNJ2 (NW_047343).

KCNJ2 3′UTR 5′. . . GCUUUUCUUUCUUUGCACAUUCCA. . . 3′
rno-miR-1 3′ UAUGUGUGAAGAAA--UGUAAGGU 5′

Note: The sequences show the unique sites of miRNA::mRNA complementarity between miR-1 and KCNJ2, for rat genes. The matched base pairs are bold and connected by a vertical line.

Data analysis

All data are presented as means ± SEM. Levels of statistical significance were assessed by Student's *t*-test or χ^2-test, as appropriate. One-way ANOVA followed by Bonferroni or Dunnett's *post hoc* test was used for multiple comparisons. A two-tailed $P < 0.05$ was considered to show a statistically significant difference. The number of experiments or animals is specified in figure legends. Data were analysed using the GraphPad Prism 5.0 and pCLAMP 8.0 software.

Materials

Tanshinone IIA was purchased from Xian Guanyu Bio-tech Co. Ltd., China. Quinidine was purchased from British Drug Houses Ltd., UK. Both of them were kept as stock solutions, in dimethyl sulphoxide (DMSO). The final concentration of DMSO did not exceed 0.1%.

Results

Effects of tanshinone IIA on arrhythmias and mortality in MI rats

The occurrence of arrhythmias was analysed firstly within 6 h post-infarction (Figure 2A). After MI, arrhythmia duration was increased (161 ± 6 s) in rats compared with those in control group (Figure 2B). Tanshinone IIA and quinidine significantly decreased the duration of arrhythmias and reduced the incidence of VT/VF in MI rats (Figure 2C, $n = 12$, $P < 0.01$). Figure 2D shows that the arrhythmia score was decreased by treatment of MI rats with tanshinone IIA or quinidine ($n = 12$, $P < 0.01$). The effects of tanshinone IIA or quinidine on the mortality of rats after MI were assessed 3 months after left anterior descending ligation. The mortality was significantly reduced in the group treated with tanshinone IIA, whereas treatment with quinidine increased mortality to about 80% ($n = 25$, $P < 0.01$, χ^2-test, Figure 2E).

Effects of Tanshinone IIA on I_{K1} current in rat cardiomyocytes

The I_{K1} plays a role in maintaining resting membrane potential (RMP) and during the late repolarizing phase of the action potential. The RMP was depolarized in cardiomyocytes in MI rats, relative to that in cardiomyocytes from control rats, indicating that I_{K1} was impaired. The depolarized RMP in MI rats was restored to control levels by tanshinone IIA. However, quinidine produced a contrary effect with the RMP being further reduced in the cardiomyocytes from quinidine-treated rats, compared with control ($n = 12$, $P < 0.01$, Figure 3A). To evaluate the role of I_{K1} at physiological RMP values, we conducted an analysis at a test potential of –90 mV (Figure 3B,C). Consistently, I_{K1} current density was reduced to -7.2 ± 0.7 pA·pF^{-1} in rat ventricular cardiomyocytes after MI,

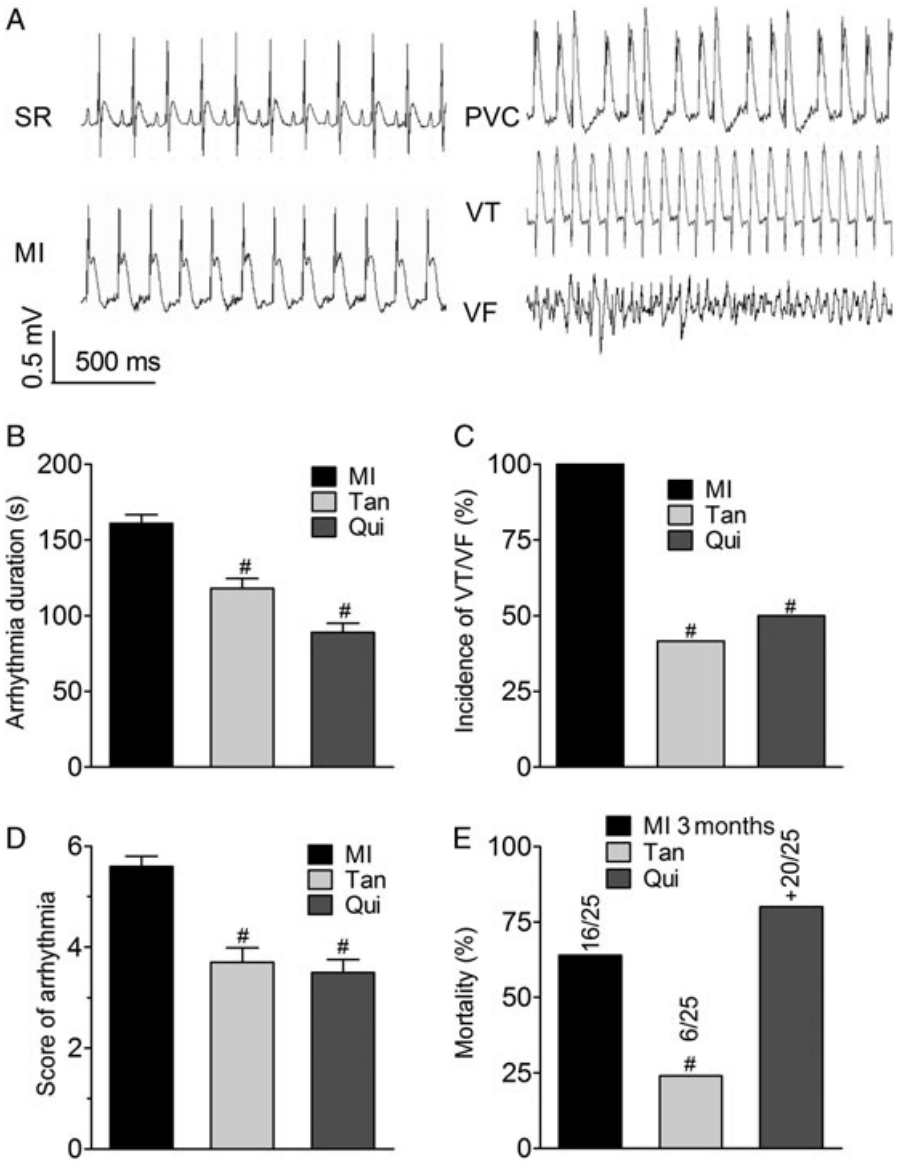

Figure 2 Effects of tanshinone IIA (Tan) and quinidine (Qui) on ischaemic arrhythmias induced by coronary artery ligation for 6 h in rats. (A) ECG recordings from different groups. Sinus rhythm (SR) and various types of ventricular arrhythmias induced by coronary artery ligation, consisting of premature ventricular contraction (PVC), ventricular tachycardia (VT) and ventricular fibrillation (VF). (B) Both Tan and Qui reduced duration of arrhythmias during myocardial infarction (MI). #$P < 0.01$ versus MI, $n = 12$ for each group. (C) Tan and Qui decreased the occurrence of ventricular tachycardia/fibrillation (VT/VF). #$P < 0.01$ versus MI ($n = 12$); χ^2-test. (D) Both Tan and Qui lowered the arrhythmia score. #$P < 0.01$ versus MI ($n = 12$). (E) Effect of Tan (10 mg·kg^{-1}·d^{-1}), or Qui (10 mg·kg^{-1}·d^{-1}) on mortality in rats 3 months after MI. #$P < 0.01$ versus MI, +$P < 0.01$ versus Tan; Data are expressed as mean ± SEM. $n = 25$, χ^2-test.

from –11.7 ± 1.1 pA·pF^{-1} in those of the control group. Along with the decreased current density, the reversal potential of I_{K1} was shifted towards depolarization, which may account for the depolarized RMP in cardiomyocytes from MI rats. Tanshinone IIA treatment restored the decreased I_{K1} at –90 mV (–9.5 ± 0.6 pA·pF^{-1}, $n = 12$, $P < 0.05$), as well as the reversal potential of I_{K1}. On the contrary, I_{K1} was further inhibited by quinidine at test potential from –120 mV to –90 mV (–5.8 ± 0.7 pA·pF^{-1} at –90 mV, $n = 12$, $P < 0.05$, Figure 3C). Moreover, quinidine had no effect on the depolarized membrane potential.

Effects of tanshinone IIA on the Kir2.1 protein and mRNA levels

To investigate the molecular basis for the changes in I_{K1} density, we examined the effects of tanshinone IIA and quinidine on expression of Kir2.1 in MI rats. More than 1.6-fold reduction of Kir2.1 protein was detected in MI hearts compared with control (Figure 4A,B, $n = 12$, $P < 0.01$). The depressed level of Kir2.1 protein in MI rat was restored by tanshinone IIA but significantly lower in the quinidine group ($n = 12$, $P < 0.01$). Kir2.1 mRNA levels had no significant difference among four groups (Figure 4C, $n = 6$, $P > 0.05$).

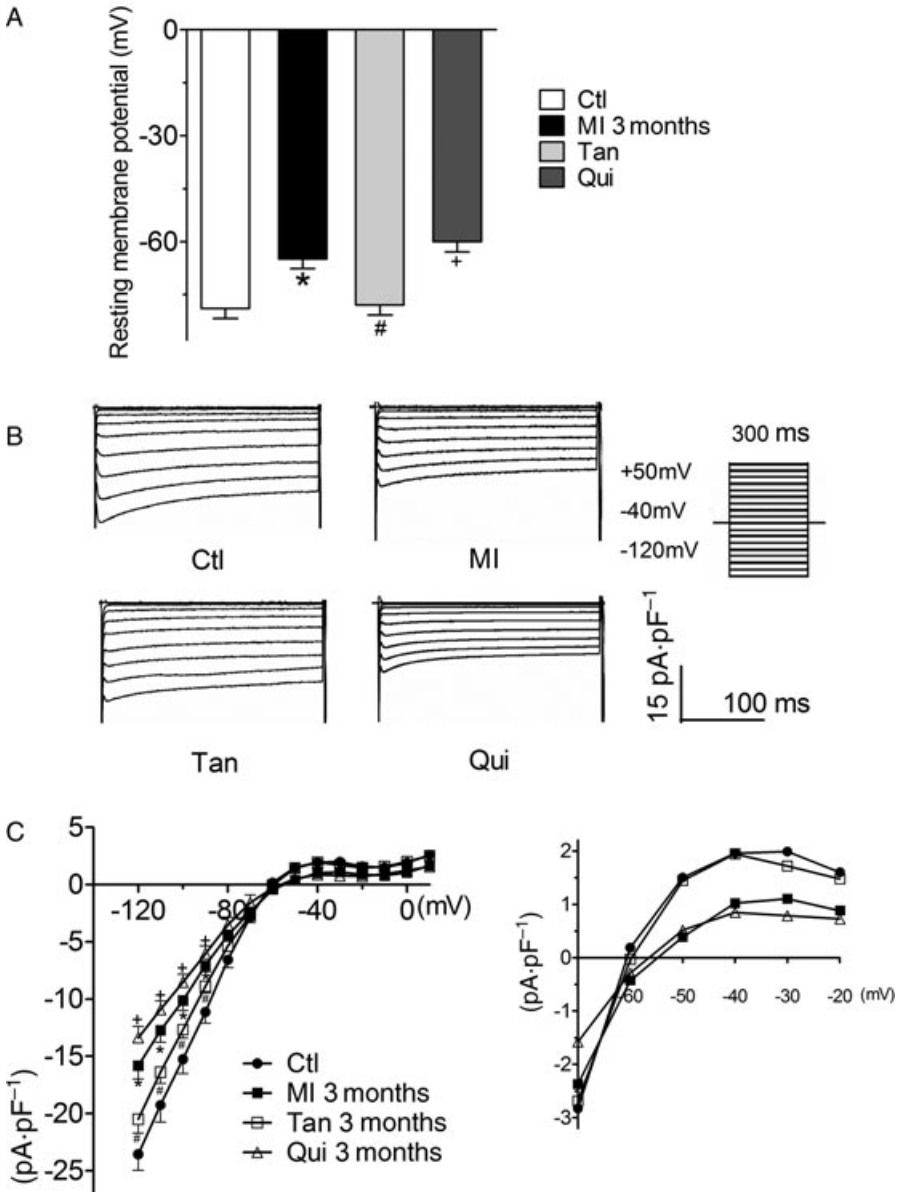

Figure 3 Effects of tanshinone IIA (Tan) and quinidine (Qui) on resting membrane potentials (RMP) and inward rectifying K^+ current (I_{K1}) in ventricular cardiomyocytes isolated from rat hearts 3 months after myocardial infarction (MI). (A) RMP was differently affected by Tan and Qui. $n = 12$, both from eight rats for each condition, $^*P < 0.01$ versus control (Ctl), $\#P < 0.01$ versus MI, $+P < 0.01$ versus Tan. (B) I_{K1} traces were recorded by whole-cell patch-clamp. (C) Mean current–voltage curves of I_{K1}. $n = 12$, $^*P < 0.05$ versus Ctl, $\#P < 0.05$ versus MI, $+P < 0.05$ versus Tan. For clarity, the outward I_{K1} at voltages between −70 mV and −20 mV is shown (on an expanded scale) in the right hand panel.

Effects of tanshinone IIA on levels of miR-1 from rat hearts 3 months after MI

MiR-1 is specifically expressed in adult cardiac and skeletal muscle tissues and is involved in pathophysiological processes relevant to coronary artery disease and ischaemic arrhythmias. The KCNJ2 gene, which encodes the inwardly rectifying potassium channel subunit Kir2.1, is a target for miR-1. To verify the involvement of miR-1 in regulation of Kir2.1 expression by tanshinone IIA, we measured the level of miR-1 in different experimental groups. We found that expression of miR-1 increased (2.9-fold) in MI rat hearts compared with control. MiR-1 expression was elevated (3.7-fold) in RNA samples isolated from rats treated with quinidine compared with control. On the contrary, the overexpression of miR-1 was reversed by tanshinone IIA in MI rats (1.1-fold, Figure 5A). Taken together, the results suggested that the overexpression of miR-1 inhibited expression of Kir2.1 protein, and that tanshinone IIA may downregulate the overexpression of miR-1 induced by long-term ischaemia, thus restoring the depressed levels of Kir2.1 protein in MI rats.

Effects of tanshinone IIA on levels of SRF in rat hearts 3 months after MI

Serum response factor, a cardiac-enriched transcription factor, is an obligate activator of miR-1 expression during

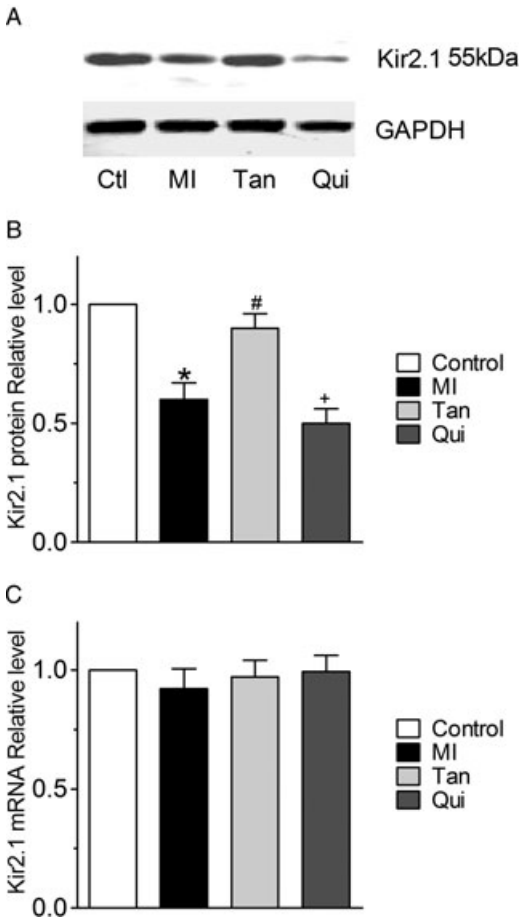

Figure 4 Effects of tanshinone IIA (Tan) and quinidine (Qui) on expression of Kir2.1 in rat hearts 3 months after myocardial infarction (MI). Membrane protein samples were extracted from the peri-ischaemic zone in rat hearts, and Kir2.1 protein levels were determined by Western blotting. (A) Examples of Western blot bands; (B) Data are mean ± SEM normalized to control hearts (Ctl). Quantitative assay was performed with GAPDH as an internal control. Kir2.1 expression level was reduced in MI rats. Tan restored the reduction of Kir2.1 protein levels ($n = 12$). Independent samples for each group. *$P < 0.01$ versus Ctl, #$P < 0.01$ versus MI, +$P < 0.01$ versus Tan. (C) Kir2.1 mRNA (KCNJ2) levels were quantified by RT real-time PCR ($n = 6$, $P > 0.05$). There was no significant difference among groups.

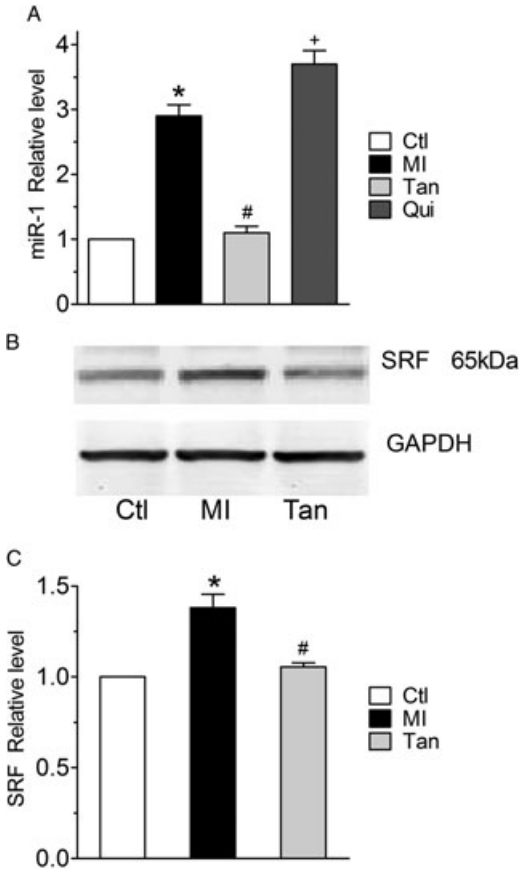

Figure 5 Effects of Tan on levels of miR-1 and SRF in hearts 3 months after MI. (A) Levels of miR-1 were quantified by RT real-time PCR ($n = 6$). Data are expressed as mean ± SEM normalized to control hearts (Ctl); *$P < 0.01$ versus Ctl, #$P < 0.01$ versus MI, +$P < 0.01$ versus Tan. (B) Effects of Tan on levels of SRF in MI rats. Examples of SRF Western blot bands. (C) Quantitative assay was performed with GAPDH as an internal control. Data are expressed as mean ± SEM normalized to Ctl; *$P < 0.01$ versus Ctl, #$P < 0.05$ versus MI. MI, myocardial infarction; Qui, quinidine; SRF, serum response factor; Tan, tanshinone IIA.

cardiac development in the mouse (Zhao *et al.*, 2005). So in this study, we have selected SRF as a target factor that may be modulated by tanshinone IIA. SRF was up-regulated in MI compared with control (1.4-fold, Figure 5B,C). Moreover, the expression of miR-1, a transcriptional target of SRF, was also robustly increased (Figure 5A). Tanshinone IIA significantly inhibited this elevated SRF expression in MI rats.

To establish the causal relationship between tanshinone IIA treatment and miR-1 up-regulation and Kir2.1 down-regulation upon MI, we also examined the miR-1 and Kir2.1 levels in a group of rats given short-term treatment with tanshinone IIA. MiR-1 expression was elevated in RNA samples isolated from rats 6 h after induction of MI, and this up-regulation of miR-1 was decreased by tanshinone IIA (Figure 6A). The protein level of Kir2.1 was a little lower in samples from MI rats than those from the control group (Figure 6B). Tanshinone IIA restored the levels of Kir2.1 protein, compared with those in MI rats. The effects of tanshinone IIA in regulating miR-1 were similar after both short-term and long-term treatment. However, tanshinone IIA had more obvious anti-arrhythmic potency in the long-term treatment and against SCD.

Discussion

Our study demonstrated a novel mechanism by which tanshinone IIA raised survival rates and reduced arrhythmias: tanshinone IIA ameliorated dysfunction of I_{K1} accompanied by a reduced overexpression of miR-1 in MI hearts. By comparison, the prototype anti-arrhythmic agent quinidine, failed to alter miR-1 levels, worsened I_{K1} dysfunction and decreased the survival rate of MI rats.

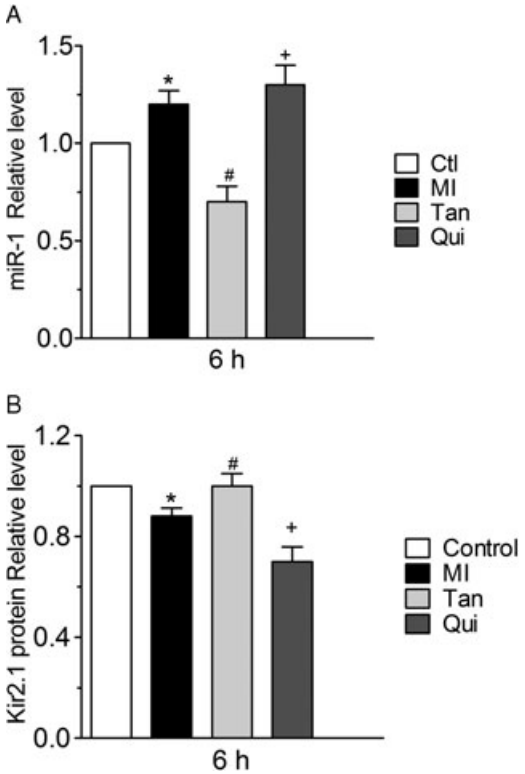

Figure 6 Effects of Tan on expression of miR-1 and Kir2.1 in rat hearts 6 h after MI. (A) Levels of miR-1 were quantified by RT real-time PCR. Data are mean ± SEM normalized to control hearts (Ctl) ($n = 6$). *$P < 0.05$ versus Ctl, #$P < 0.01$ versus MI, +$P < 0.01$ versus Tan. (B) Kir2.1 protein levels were determined by Western blot with GAPDH as an internal control. ($n = 6$).*$P < 0.05$ versus Ctl, #$P < 0.05$ versus MI, +$P < 0.05$ versus Tan. MI, myocardial infarction; Qui, quinidine; Tan, tanshinone IIA.

Tanshinone IIA reduced cardiovascular mortality in post-MI rats by reducing arrhythmias. A heart attack (MI) occurs when there is a blockage in one or more of the coronary arteries, which reduces blood flow through the coronary arteries, or normal myocardial perfusion is arrested preventing the heart from receiving enough oxygen-rich blood. MI results in myocardial ischaemia, necrosis and induces ventricular arrhythmias. An earlier MI is an important risk factor for SCD, due primarily to ventricular tachyarrhythmias (Clements-Jewery *et al.*, 2005). Often after MI, the surviving heart muscle hypertrophies and simultaneously undergoes an electrical remodelling of membrane ion channels, which favours arrhythmia. Arrhythmias are electrical disturbances that can result in irregular heart beating with consequent insufficient pumping of blood, which can lead to SCD. Tanshinone IIA decreased the duration of arrhythmias, the incidence of VT/VF and thereby cardiac death.

I_{K1} is a strong inward rectifying K^+-selective current, which plays an important role in shaping the normal action potential and modulates the RMP and the final repolarization phase in cardiomyocytes (Carmeliet, 1999; Jones, 2003; Diaz *et al.*, 2004; Chilton *et al.*, 2005). Studies show that I_{K1} plays an important role in ventricular arrhythmias, highlighted by the recently described Andersen's syndrome and studies in the guinea pig heart model of VF (Dhamoon and Jalife, 2005). Decreased I_{K1} is present after infarction in rat and rabbit hearts (Liu *et al.*, 2004; Yang *et al.*, 2007a). The down-regulation of I_{K1} produces depolarization of the RMP and prolongation of the APD. When I_{K1} is decreased, early after-depolarizations become inducible and a decreased I_{K1} is one of the important reasons for the promotion of arrhythmias induced by delayed after-depolarization. The down-regulation of I_{K1} may also predispose to QT prolongation and ventricular arrhythmias. Thus dysfunction of I_{K1} can cause electrophysiological disturbances and increase the risk for life-threatening arrhythmias and SCD. Our findings demonstrated that tanshinone IIA restored the decreased I_{K1} rat ventricular myocytes after MI. This I_{K1} dysfunction in MI hearts was caused by functional impairment and down-regulation of expression of Kir2.1, the pore-forming α-subunit of the potassium channel carrying I_{K1}. Tanshinone IIA restored the expression of Kir2.1 protein level that had been inhibited after MI. Sun *et al.* (2008) reported tanshinone IIA activated the human cardiac KCNQ1/KCNE1 potassium channels (carrying I_{Ks}), but not the inward rectifier potassium channels (carrying I_{K1}). They found that tanshinone IIA had no direct action on I_{K1} in HEK 293 cells. However, in our experiments, tanshinone IIA was given long term (3 months) and *in vivo* to MI rats, and our results showed that tanshinone IIA indirectly regulated the I_{K1} by restoring the expression of Kir2.1 through effects on miR-1. Myocardial ischaemia induces perturbations in ion channel homeostasis, and it is likely that tanshinone IIA modulates the imbalance of ion channels rather than affecting a single ion channel, thus preventing the electrophysiological disorders associated with ischaemia.

The discovery of miRNA may help us further understand heart diseases. MiR-1 has been conserved from Drosophila (Kwon *et al.*, 2005; Nguyen and Frasch, 2006) to humans. MiR-1 plays an essential role in pathophysiological functions in the heart and is a potential anti-arrhythmic target (Kwon *et al.*, 2005; Zhao *et al.*, 2005). MiR-1 has many target genes encoding cardiac ion channels, such as GJA1, KCND2 and KCNJ2 that respectively encode connexin-43, Kv4.2 and Kir2.1 (Nakamura *et al.*, 1998; Leonoudakis *et al.*, 2004). The 3′-UTRs of the KCNJ2 gene contain stretches of eight nucleotides that are complementary to the first eight nucleotides from the 5′ end of miR-1. MiR-1 might thus post-transcriptionally repress genes such as KCNJ2 to regulate the levels of Kir2.1 protein (Yang *et al.*, 2007a). Our present study showed that expression of miR-1 was clearly increased in ventricular myocytes at 3 months post-infarction, and expression of Kir2.1 was similarly decreased, consistent with the decrease of I_{K1}, accompanied by exacerbated arrhythmogenesis. Importantly, Kir2.1 mRNA levels were not significantly different among four groups, implying that miR-1 had no significant effect on Kir2.1 mRNA levels, but that it post-transcriptionally repressed KCNJ2 to regulate Kir2.1 protein level. Overexpression of miR-1 could therefore lead to a reduction in I_{K1} current, a slowing of conduction and depolarization of the RMP. Tanshinone IIA significantly inhibited this overexpression of miR-1, and it is possible that the restored RMP (after tanshinone IIA) was better able to resist arrhythmias. Our data showed that miRNA-1 was significantly down-regulated in MI rats treated with tanshinone IIA for 3 months. Tanshinone IIA may, by direct or indirect effects, control miR-1 levels. Studies showed tanshinone IIA depressed cardiomyocyte hypertrophy through the

MEK/ERK pathway and inhibited the activation of the promoter of human α1 collagen gene (Zhang *et al*., 2006a; Yang *et al*., 2007b). This suggests that tanshinone IIA may take part in post-transcriptional regulation or by affecting transcriptional factors, via various signalling pathways, may change miR-1 expression.

Serum response factor is a transcriptional regulator of numerous muscle-specific and growth-regulated genes, modulating the balance between muscle proliferation and cell migration and proliferation (Miano *et al*., 2004; Wang *et al*., 2004). SRF may also direct the expression of microRNAs (miRs) that inhibit the expression of cardiac regulatory factors. SRF and cofactors play an obligatory role in cardiogenesis by driving the expression of silencer miRNA (Niu *et al*., 2007). Mouse miR-1-1 and miR-1-2 were regulated by SRF (Kwon *et al*., 2005), and cardiac miR-1 levels are adjusted directly by SRF (Zhao *et al*., 2005; Niu *et al*., 2007). We found tanshinone IIA to inhibit the increased level of SRF in MI rat hearts, and this could be one of mechanism by which tanshinone IIA reduced miR-1 expression.

Quinidine is one of the most commonly used drugs for treatment of both atrial and ventricular rhythm disturbances (Grace and Camm, 1998). Quinidine's ability to slow autorhythmicity, conduction and prolongation of repolarization has been attributed to its ability to inhibit several types of ion channels in heart, including sodium, calcium and potassium channels. Quinidine has moderate efficacy in patients with sustained ventricular arrhythmias. Clinically, particularly in patients with previous MI, quinidine increases the risk of arrhythmias (Grace and Camm, 1998). We found that both of tanshinone IIA and quinidine suppressed arrhythmias during the early MI (short-term). While tanshinone IIA reduced long-term ischaemic mortality, quinidine increased mortality to about 80% in MI rats after 3 months, suggesting that tanshinone IIA would have better results in long-term maintenance therapy. In our study, quinidine had no effect on the overexpressed miR-1 during MI, and the depressed I_{K1} induced by MI was further reduced by quinidine. The present study revealed the ability of tanshinone IIA to diminish the lethal arrhythmias during MI and the associated mortality rate via down-regulation of miR-1 expression. Our results have expanded our understanding of anti-arrhythmic therapy, supporting the view that miRNAs are likely to have important functions and may be novel therapeutic targets.

In summary, our findings showed that tanshinone IIA had better cardioprotective effects on ischaemic arrhythmias compared with quinidine. Moreover, down-regulation of the overexpressed miR-1 level in MI hearts by tanshinone IIA may contribute to the reduction in mortality. These studies show that tanshinone IIA modulates the overexpressed miR-1 by regulating SRF, which may play a role in decreasing the risk of SCD.

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Conflict of interest

The authors state no conflict of interest.

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