MicroRNA-1 downregulation by propranolol in a rat model of myocardial infarction: a new mechanism for ischaemic cardioprotection

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Aims The present study was designed to investigate whether the beneficial effects of β-blocker propranolol are related to regulation of microRNA miR-1.

Methods and results We demonstrated that propranolol reduced the incidence of arrhythmias in a rat model of myocardial infarction by coronary artery occlusion. Overexpression of miR-1 was observed in ischaemic myocardium and strikingly, administration of propranolol reversed the up-regulation of miR-1 nearly back to the control level. In agreement with its miR-1-reducing effect, propranolol relieved myocardial injuries during ischaemia, restored the membrane depolarization and cardiac conduction slowing, by rescuing the expression of inward rectifying Kþ channel subunit Kir2.1 and gap junction channel connexin 43. Our results further revealed that the β-adrenoceptor–cAMP–Protein Kinase A (PKA) signalling pathway contributed to the expression of miR-1, and serum response factor (SRF), which is known as one of the transcriptional enhancers of miR-1, was up-regulated in ischaemic myocardium. Moreover, propranolol inhibited the β-adrenoceptor–cAMP–PKA signalling pathway and suppressed SRF expression.

Conclusion We conclude that the β-adrenergic pathway can stimulate expression of arrhythmogenic miR-1, contributing to ischaemic arrhythmogenesis, and β-blockers produce their beneficial effects partially by down-regulating miR-1, which might be a novel strategy for ischaemic cardioprotection.

KEYWORDS Propranolol; Arrhythmia; MicroRNA; Ion channels; Myocardial infarction

1. Introduction

Ischaemic heart disease is a leading cause of morbidity and mortality globally and the associated financial burden is increasing every year.1,2 Myocardial infarction (MI), a condition in which normal myocardial perfusion is arrested, is characterized by a loss of excitability followed by cascades of ionic, functional, and metabolic abnormalities, which can induce arrhythmias and sudden cardiac death. Many clinical trials with antiarrhythmic drugs (sodium-channel blockers, potassium-channel blockers, calcium-channel blockers) failed to prevent cardiac death triggered by malignant arrhythmias in patients with MI3–5; moreover, at least Naþ channel-blocking antiarrhythmic agents increased mortality.6–8 Fortunately, β-adrenoceptor blockers have been demonstrated to produce cardioprotective effects in several studies; however, the essential mechanism remained unclear. β-Adrenergic blockers have widely been used in the clinic for the treatment of cardiovascular diseases such as hypertension, coronary heart disease, and hyperlipidaemia, especially for preventing sudden cardiac death in patients suffering acute and chronic MI.6–8 These agents have been shown to produce a wide variety of effects, including improvement of coronary circulation, reduction of infarct size, protection from cardiomyocyte apoptosis, prevention of Ca2þ overload, scavenging of oxygen-free radicals, and amelioration of cellular metabolism.8–10 The beneficial effects of propranolol involve altered gene expression upon β-adrenoceptor blockade, but the subsequent mechanisms remain poorly understood.

miRNAs11 emerged as a critical node in post-transcriptional regulation of growth, apoptosis, and other cellular processes within almost all cells and as one of the most important and versatile regulators at the core of human physiology and disease. A recent study demonstrated

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that miR-21 regulates MMP-2 expression in cardiac fibroblasts of the myocardial infarct zone through a PTEN pathway. We have established the pathological roles of the muscle-specific miRNAs miR-1 and miR-133 in ischaemic myocardial injuries, cardiomyocyte apoptosis, and diabetic cardiomyopathy. Terentyev et al. demonstrated that altered Ca$^{2+}$ signalling presents another potential arrhythmogenic mechanism for miR-1. While these findings suggest miR-1 to be a potential target for anti-ischaemic arrhythmia therapy, whether inhibiting miR-1 can offer ischaemic cardioprotecting outcomes has not been tested.

The aim of the present study was to complement and extend our earlier studies by investigating how miR-1 expression is up-regulated and if the beneficial effects of propranolol are related to miR-1 expression in a rat model of MI. To this end, we evaluated the effects of propranolol on arrhythmia incidence and the underlying electrophysiological mechanisms, and the effects of propranolol on expression of miR-1 and SRF, an important transcriptional factor in cardiac cells that is known to be an enhancer of miR-1 transcription. We also exploited the role of the β-adrenoceptor-CAMP-dependent Protein Kinase A (PKA) signalling pathway in the regulation of miR-1 expression in MI rats.

2. Methods
An online supplement is available with expanded methods.

2.1 Animals
Male Wistar rats (250–300 g) were used in the present study. Use of animals was in accordance with the 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).

2.2 Drugs
Propranolol (Sigma-Aldrich, St Louis, MO, USA) and isoproterenol (Sigma-Aldrich) were used in the current study (see Supplementary material online).

2.3 Rat model of MI
Wistar rats initially anesthetized with pentobarbital (40 mg/kg, i.v.) were randomly divided into six groups: control, isoproterenol (ISO), ischaemia (MI), ischaemia–propranolol (MI–PRO), ischaemia–propranolol–miR-1 (MI–PRO–miR-1) and ischaemia–AMO (2-0-methyl-modified antisense oligonucleotides)-1 (MI–AMO-1). Subcutaneous injection of isoproterenol (10 mg/kg) at the back of the rats was performed to activate β-adrenoceptor in the intact heart. Propranolol was administrated 7 days before the experiments with daily oral doses of 10 mg/kg (1, 5, 10, 50 mg/kg for dose-dependent study, n = 7 for each group) (see Supplementary material online).

2.4 In vivo gene transfer of miR-1 and AMO-1
AMO-1 contains 2'-O-methyl modifications at every base and a 3'-C3-containing amino linker. The synthesized AMO-1 has been confirmed to have the ability of inhibiting miR-1 function, and was delivered into the infarcted myocardium by in vivo gene transfer as described previously (see Supplementary material online).

2.5 Measurement of infarct size
Size of the infarcted area was estimated by the volume and weight as a percentage of the left ventricle (see Supplementary material online).

2.6 Measurement of haemodynamic function
Haemodynamic function was assessed before and 12 h after infarction by determining left ventricular end-diastolic pressure (LVEDP), and time derivatives of pressure were measured during contraction (±dP/dt) and relaxation (−dP/dt) recorded on a polygraph.

2.7 Measurement of arrhythmias
The standard limb lead II ECG was recorded when the rats were anesthetized for 1 h before and 11 h after coronary artery ligation. The incidence of arrhythmias were registered and evaluated in accordance with the Lambeth Conventions (see Supplementary material online).

2.8 Quantification of miR-1 levels
The mirVana™ qRT-PCR miRNA Detection Kit (Ambion) is a quantitative RT-PCR kit enabling sensitive, rapid quantification of miRNA expression from total RNA samples, was used in conjunction with real-time PCR with SYBR Green I for quantification of miR-1 transcripts, following the manufacturer’s instructions (see Supplementary material online).

2.9 Transmission electron microscopy
Selected samples were fixed in 2.5% glutaraldehyde in 0.1 mol/L PBS (pH 7.4). The specimens were then rinsed in buffer, post-fixed in PBS-buffered 1% OsO4 for 1–2 h, stained en bloc in uranyl acetate, dehydrated in ethanol, and embedded in epoxy resin by standard procedures. The ultra-thin sections were electron stained and observed under an electron microscope (JEM-1220, JEOL Ltd., Tokyo, Japan).

2.10 Myocyte isolation and whole-cell patch-clamp techniques
The myocyte dissociation procedure was similar to that described previously. 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 (see Supplementary material online).

2.11 Western blot analysis
Western blots were probed with the following primary antibodies: Cx43, Kir2.1, PKA, and SRF (Santa Cruz). Western blot bands were quantified using Odyssey v1.2 software by measuring the band intensity (area × OD) for each group and normalizing to GAPDH (anti-GAPDH antibody from Kangcheng Inc.) as an internal control (see Supplementary material online).

2.12 Determination of cAMP content
125-I-CAMP RIA Kit was provided by the nuclear medicine laboratory of Shanghai university of Traditional Chinese Medicine. Auto-gamma counter was used to measure the cpm values. The content of cAMP was determined according to the manufacturer’s protocol. Experiments were performed at room temperature, and data were relative to the control group.
2.13 Preparation and transfection of decoy ODNs
Single stranded phosphorothioate decoy oligodeoxynucleotides (ODNs) were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd (Shanghai), and transfected with lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) (see Supplementary material online).

2.14 Luciferase activity assay
For luciferase assay involving analysis of miR-1 promoter activities, HEK293 cells were transfected with 1 μg PGL3-target DNA (firefly luciferase vector) and 0.1 μg PRL-TK (TK-driven Renilla luciferase expression vector) with lipofectamine 2000 (see Supplementary material online).

2.15 Data analysis
Group data are expressed as mean ± SEM and analysed by GraphPad Prism 5.0, pCLAMP 8.0, 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 follow the beneficial effects. Administration with propranolol in a dose-dependent fashion (Figure 2A) shows the protective effect of propranolol against arrhythmias induced by coronary artery ligation in rats. We found that miR-1 expression was elevated (~1.5-fold) in the isoproterenol-injected healthy rats, compared with non-injected hearts (Figure 2A). To clarify that β-adrenergic receptor activation with isoproterenol directly induces miR-1 expression, we performed additional experiments in cultured cardiomyocytes. miR-1 expression was significantly up-regulated (~16-fold) after treatment with 1 μM isoproterenol for 4 h in neonatal rat ventricular cells (see Supplement material online, Figure S1). The result confirmed direct stimulation of β-adrenoceptor causes much higher expression of miR-1 in cultured cardiomyocytes without secondary stress events involvement. Consistent with our previous observations, 12 h MI resulted in around three-fold increase in the level of miR-1. This up-regulation of miR-1 expression was abrogated in animals administered with propranolol in a dose-dependent fashion (Figure 2A).

3. Results
3.1 Protection of ischaemic hearts by propranolol and miR-1 antisense oligonucleotide
Table 1 shows the haemodynamic parameters before and after administration of propranolol after occlusion, and the effects of exogenous miR-1 on these parameters. Myocardial infarction caused significant increases in LVEDP as well as reduction of +dP/dt, indicating impaired cardiac systolic function. Administration with propranolol or transfection of AMO-1 ameliorated these deleterious changes; LVEDP was markedly reduced, and the decreases in +dP/dt measured in the infarcted rats were diminished. Strikingly, transfection of exogenous miR-1 offset the beneficial effects.

Table 1: Effects of propranolol, miR-1, and AMO-1 on haemodynamic parameters during MI

<table>
<thead>
<tr>
<th>Group</th>
<th>HR (b.p.m.)</th>
<th>LVEDP (mmHg)</th>
<th>+dP/dt (mmHg/s)</th>
<th>−dP/dt (mmHg/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>338 ± 36</td>
<td>3.7 ± 1.0</td>
<td>3418 ± 283</td>
<td>2988 ± 668</td>
</tr>
<tr>
<td>MI</td>
<td>359 ± 40</td>
<td>14.0 ± 2.5*</td>
<td>2751 ± 381*</td>
<td>2097 ± 326*</td>
</tr>
<tr>
<td>MI-PRO</td>
<td>339 ± 38</td>
<td>7.0 ± 1.8*</td>
<td>3147 ± 103</td>
<td>2847 ± 67*</td>
</tr>
<tr>
<td>MI-PRO+miR-1</td>
<td>358 ± 34</td>
<td>12.5 ± 1.0*</td>
<td>2831 ± 105†</td>
<td>2210 ± 229†</td>
</tr>
<tr>
<td>MI-AMO-1</td>
<td>349 ± 39</td>
<td>8.6 ± 2.0*</td>
<td>3099 ± 124*</td>
<td>2782 ± 324*</td>
</tr>
</tbody>
</table>

HR, heart rate; LVEDP, left ventricular end-diastolic pressure; dP/dt, time derivatives of pressure. All values are mean ± SD. One-way ANOVA followed by Bonferroni’s post-hoc test. n = 7 in each group.

3.2 Regulation of miR-1 expression by β-adrenergic agonist and blocker in healthy and ischaemic hearts
miR-1 is considered a muscle-specific miRNA, and it plays an important role in ischaemic arrhythmogenesis. Here, we determined the effects of β-adrenergic antagonist on expression of miR-1 by qRT-PCR. To identify whether activation of β-adrenergic receptor in the intact heart can directly increase miR-1 expression, we performed subcutaneous injection of isoproterenol (10 mg/kg) at the back of the rats to activate β-adrenoceptor in the intact heart of rat. We found that miR-1 expression was elevated (~1.5-fold) in the isoproterenol-injected healthy rats, compared with non-injected hearts (Figure 2A). To clarify that β-adrenergic receptor activation with isoproterenol directly induces miR-1 expression, we performed additional experiments in cultured cardiomyocytes. miR-1 expression was significantly up-regulated (~16-fold) after treatment with 1 μM isoproterenol for 4 h in neonatal rat ventricular cells (see Supplement material online, Figure S1). The result confirmed direct stimulation of β-adrenoceptor causes much higher expression of miR-1 in cultured cardiomyocytes without secondary stress events involvement. Consistent with our previous observations, 12 h MI resulted in around three-fold increase in the level of miR-1. This up-regulation of miR-1 expression was abrogated in animals administered with propranolol in a dose-dependent fashion (Figure 2A).

3.3 Normalization of expression of key cardiac ion channel proteins by propranolol
Our previous study has established GJA1 (encodes Cx43) and KCNJ2 (encodes Kir2.1) as targets of miR-1. The gap junctions were clearly located on the site of intercalated discs, normal myocardium was characterized by intact nucleus and mitochondria in control hearts under a transmission electron microscope. In MI hearts, in contrast, morphologically apparent gap junction ultrastructures were rarely observed in intercalated discs, which displayed focal expansion, cytoplasm had severe oedema, and the myofibrils and Z-bands were distorted. Cardiomyocytes contain vacuoles, numerous damaged mitochondria with loss of electrodense matrix, and amorphous matrix densities in mitochondria. Propranolol...
reduction was reversed by propranolol, in contrast, the recording demonstrated that the current density of IK1 was markedly decreased in ventricular myocytes isolated from MI hearts. The depression of IK1 was prevented in the cells from the animals treated with propranolol, and the current density was depressed in cells from the animals co-applicated with exogenous miR-1 (Figure 2E).

3.4 Potential role of cAMP-PKA signalling pathway and SRF in miR-1 reduction by β-adrenergic blockade

Upon stimulation of β-adrenoceptors, cAMP level is increased which can activate PKA. The latter can be translocated into nucleus where it can subsequently phosphorylate cAMP-responsive element binding protein (CREB) to activate this transcription factor which in turn can cause activation or repression of gene transcription. We investigated whether the cAMP-PKA signalling pathway could stimulate miR-1 expression and whether propranolol could down-regulate miR-1 expression by inhibiting the cAMP-PKA signalling pathway through blocking β-adrenoceptors in a rat model of MI. Our results indeed provided the evidence in support of this notion. As shown in Figure 3A, cAMP content was elevated by two-fold at 30 min after MI, accompanied by parallel up-regulation of miR-1 expression. As expected, propranolol lowered down the cAMP level back to the normal range. Furthermore, western blot analysis showed a significant up-regulation of PKA (~1.5-fold) in MI, and this up-regulation was also prevented by propranolol (Figure 3B).

To confirm that β-adrenoceptor–cAMP–PKA pathway is involved in regulation of miR-1, we treated the neonatal rat ventricular myocytes with forskolin, a cAMP activator, and found that it elevated the level of miR-1 by ~1.6-fold. The effect of forskolin was abolished by PKA inhibitor H-89 (Figure 3C).

We further identified two putative cAMP-responsive element binding protein (CREB) recognition sites within the 3300 bp region upstream to pre-miR-1 (see Supplementary material online, Figure S2). We set out to evaluate the role of the CREB in regulating transcription of miR-1 gene. To this end, we inserted a 324 bp fragment (spanning 3215 to 3539 bp region upstream to pre-miR-1) into the luciferase reporter vector, which contains two putative CREB sites. Co-transfection of the vector with the decoy oligodeoxynucleotide (ODN) inhibitors24 that contain the perfect binding sites for CREB to sequestrate these transcription factors caused substantial reduction of luciferase activities (Figure 3D). In cultured neonatal rat myocytes, transfection with the decoy ODN for CREB significantly diminished the miR-1 level (Figure 3E), indicating a role of CREB in activating the transcription of miR-1 gene.

It has been shown that expression of miR-1 is dependent upon binding of SRF to its promoter regions23,25, an important transcriptional factor in cardiovascular system.26,27 In our previous study, we demonstrated that incubation of neonatal rat cardiomyocytes with distamycin A, selectively inhibiting binding of SRF to its cis-element, largely suppressed miR-1 expression. This effect was further confirmed by silencing of SRF using the siRNA directed against SRF (SRF siRNA).15 We examined whether SRF participated in the protective effects of propranolol in MI hearts. As shown in Figure 4A, SRF protein level was found increased in MI hearts (~1.6-fold) relative to healthy hearts, and propranolol largely inhibited the increase in SRF expression. To verify SRF is directly regulated by β-adrenoceptor...
modulation, we exploited the relationship between β-adrenoceptor stimulation and SRF expression in cultured neonatal cardiomyocytes. We observed that β-adrenergic agonist isoproterenol up-regulated SRF expression by 1.5-fold, while propranolol repressed the protein level of SRF by 70%, and the result was consistent with elevated miR-1 level after stimulation of β-adrenoceptor (Figure 4B).

4. Discussion

The present study yielded several novel findings. First, we demonstrated, for the first time, that knockdown of miR-1 level or down-regulation of miR-1 expression could produce cardioprotective effects in MI rats. Secondly, the β-adrenoceptor–cAMP–PKA signalling pathway positively regulates miR-1 expression. Third, β-adrenergic blocker propranolol produce protective effects against ischaemic arrhythmias, possibly partially by reducing miR-1 level via inhibiting the β-adrenoceptor–cAMP–PKA pathway and SRF (summarized in Figure 5). These findings not only help us understand the mechanisms underlying the ischaemic cardioprotective effects of β-blockers but also conceptually advance our view of miRNAs that may serve as potential therapeutic and drug targets.

There are important fundamental implications of our findings. MI results in a dramatic disorder of automaticity, an increased heterogeneity of conduction, neurohumoral changes, structural remodelling, and alterations in gap junction and ion channel protein expression and distribution (electrical remodelling),18 all of which contribute to the development of electrical disturbance that leads to life-threatening cardiac arrhythmias. In this study, administration of propranolol significantly diminished the incidence of arrhythmias in the first 12 h of MI, which corresponds to
Propranolol down-regulate a proarrhythmic microRNA

the peri-infarction period during which phase II ischaemic arrhythmias often occur. Consistent with our previous study, 13 individuals surviving from MI presented depolarized resting membrane potential, decreased Ik1 density, loss of normal distribution of gap junctions, and down-regulation of Cx43 protein. KCNJ2 encodes Kir2.1, the main K+ channel subunit that mediates the cardiac inward rectifier K+ channels, and is responsible for setting and maintaining the cardiac resting membrane potential. 29 GJA1 encodes Cx43, the main cardiac gap junction channel that is responsible for intercellular conductance in the ventricles. 30 In particular, cell-to-cell electrical uncoupling of cardiomyocytes plays an important role in arrhythmogenesis during ischaemic heart disease due to interruption of cardiac conduction. 31 KCNJ2 and GJA1 are established target genes for miR-1 which is overexpressed in patients with coronary artery disease, and repression of these genes by miR-1 contributes significantly to the arrhythmogenic potential of miR-1 during MI. 13 Propranolol, the classical β-adrenoceptor antagonist, has widely been used in clinic for the treatment of patients at high-risk sudden cardiac death, including those with angina pectoris, coronary heart disease, MI, or heart failure. 32,33 Although the 2005 COMMIT (ClOpidogrel and Metoprolol in Myocardial Infarction Trial) 34 provided negative results of life-saving efficacy of β-blockers, several other studies revealed that β-blockers are able to produce a wide variety of beneficial effects, including improvement of circulation, reduction of infarct size, protection from cardiomyocyte apoptosis, prevention of Ca2+ overload, scavenging of oxygen-free radicals, and amelioration of cellular metabolism. 35–37 Here, we demonstrate that propranolol dose-dependently suppressed up-regulation of miR-1 and restored the impaired KCNJ2 and GJA1 gene expression and function. These changes may be a novel molecular and cellular mechanism underlying the ischaemic cardioprotective effects of the β-blockers.

Another important finding of this study is that PKA signalling pathway is involved in the regulation of expression of miR-1. Our data showed that application of forskolin, a cAMP activator, to cultured neonatal rat ventricular cells remarkably elevated the level of miR-1, an effect abolished by PKA inhibitor H-89. It is therefore expected that the β-adrenoceptor blocker, propranolol, down-regulated miR-1 expression in ischaemic heart. In line with this, we have computationally searched the 5′ flanking region of the miR-1 gene using the MatInspector program and identified two perfect putative CRE element (binding site for CREB, CGTCA) upstream of the primary miR-1 sequence. Our finding is important because augmentation of the β-adrenoceptor-cAMP-PKA signalling pathway is common in many pathological conditions of the heart and there is a possibility that miR-1 may also take a part in these conditions. This finding thus revealed a novel molecular mechanism that regulates miR-1 expression.
It has been shown that expression of miR-1 is stimulated upon binding of SRF to its promoter regions. Activation of PKA is able to phosphorylate variety of proteins, including SRF. Our results demonstrated that SRF level was significantly increased during MI and this increase was suppressed by propranolol. Inhibition of SRF may well be another mechanism by which propranolol reduce miR-1 expression. Similar results were obtained with another β-adrenoceptor blocker metoprolol (data not shown).

It has been shown in several recent studies that miR-1 plays a critical role in different pathological conditions. Ikeda et al. reported miR-1 attenuated cardiomyocyte hypertrophy by application of in vivo and in vitro models. By comparison, van Rooij et al. demonstrated there is no expression change of miR-1 in a mouse model of cardiac hypertrophy. In contrast, Terentyev et al. showed miR-1 promotes cardiac arrhythmogenesis by inhibiting expression of the PP2A regulatory subunit B56β; together with the findings of the present study, this would indicate miR-1 as an arrhythmogenic factor. Divergent effects of miR-1 on cardiomyocytes and heart functions are likely due to miR-1 can regulate multiple target genes which are involved in different pathophysiological conditions. The divergent results also reflect differences in research models or myocardial regions sampled. Taken together, this would highlight the ambivalent role of miR-1 in cardiac pathophysiology.

To summarize, our current insufficient understanding of the mechanisms underlying ischaemic arrhythmogenesis and valid antiarrhythmic targets are two of the major challenges to cardioprotection during MI. The present study revealed the ability of β-adrenoceptor blocker to diminish the lethal arrhythmias during MI that involves regulation of miR-1 expression through inhibiting its transcriptional factors. It thus expanded our understanding of antiarrhythmic therapy, reconsolidating the view that miRNAs likely have important functions and may be novel therapeutic and drug targets. It should be aware that our studies were performed in animal models and the findings may not be extrapolated directly to humans. This calls for a further precaution applying the results of this study to patients. Nevertheless, the findings open the door for further studies to investigate whether the miR-1 mechanism of β-blocker actions also operate in the clinical setting.

**Supplementary material**
Supplementary material is available at *Cardiovascular Research* online.

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References