



I_{K1} channel agonist zacopride suppresses ventricular arrhythmias in conscious rats with healing myocardial infarction

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ABSTRACT

Aims: Arrhythmogenesis of chronic myocardial infarction (MI) is associated with the prolongation of action potential, reduction of inward rectifier potassium (I_{K1} , Kir) channels and hyper-activity of Calcium/calmodulin-dependent kinase II (CaMKII) in cardiomyocytes. Zacopride, a selective I_{K1} agonist, was applied to clarify the cardioprotection of I_{K1} agonism via a CaMKII signaling on arrhythmias post-MI.

Methods: Male SD rats were implanted wireless transmitter in the abdominal cavity and subjected to left main coronary artery ligation or sham operation. The telemetric ECGs were monitored per day throughout 4 weeks. At the endpoint, isoproterenol (1.28 mg/kg, *i.v.*) was administered for provocation test. The expressions of Kir2.1 (dominant subunit of I_{K1} in ventricle) and CaMKII were detected by Western-blotting.

Key findings: In the telemetric rats post-MI, zacopride significantly reduced the episodes of atrioventricular conduction block (AVB), premature ventricular contraction (PVC), ventricular tachycardia (VT) and ventricular fibrillation (VF), without significant effect on supraventricular premature contraction (SPVC). In provocation test, zacopride suppressed the onset of ventricular arrhythmias in conscious PMI or sham rats. The expression of Kir2.1 was significantly downregulated and p-CaMKII was upregulated post-MI, whereas both were restored by zacopride treatment.

Significance: I_{K1} /Kir2.1 might be an attractive target for pharmacological controlling of lethal arrhythmias post MI.

1. Introduction

Cardiac arrhythmias are frequently concurrent in many cases of cardiovascular abnormalities, such as coronary atherosclerosis, myocardial infarction (MI), ischemic and dilated cardiomyopathies, congestive heart failure (HF), hyperlipidemia, diabetes mellitus and hypertension. Of which, acute MI is a leading cause of sudden cardiac death due to the occurrence of lethal ventricular arrhythmias including ventricular tachycardia (VT), ventricular fibrillation (VF) and cardiac arrest. Patients confront arrhythmias not only in acute MI, but also in

late (healing or healed) phase. Even survived from acute MI, about 50% of patients died of fatal ventricular arrhythmias [1]. Sympathetic nerve activation, as may occur in some stress events, e.g., cold exposure, exercise [2,3], sports matches [4] or episodes of anger [5], alters cardiac electrophysiology and render individuals with ischemic heart disease much higher risk of sudden cardiac death.

Antiarrhythmic drugs have been subdivided into four classes plus a group of miscellaneous agents that cannot be classified into any of the four classes, such as digoxin and adenosine [6,7]. The classification is mainly based on their effects on cardiac action potentials owing to their

Abbreviations: MI, myocardial infarction; AP, action potential; VT, ventricular tachycardia; VF, ventricular fibrillation; CaMKII, calcium/calmodulin-dependent protein kinase II; DAD, delayed afterdepolarization

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blocking effects on Na⁺ channels (class I), β-receptors (class II), K⁺ channels (class III) and Ca²⁺ channels (class IV) [8]. However, CAST study [9] has revealed the potential proarrhythmic risks of Na⁺ channel blockers. Blockers of K⁺ channels and Ca²⁺ channels also have the potential proarrhythmic side effects [10,11]. Therefore, it is necessary to develop new type(s) of antiarrhythmic drugs based on the comprehensive understanding of drug actions and new antiarrhythmic mechanisms. Cardiac inward rectifier potassium channels (I_{K1}), which present in all atrial and ventricular myocytes, create the main conductance that controls the resting potential and permits a significant repolarizing current during the terminal phase of action potential [12,13]. Agonists or openers of I_{K1} channels may have profound effects on cardiac excitability and theoretically antiarrhythmic benefit. Zocopride is the only reported selective I_{K1} channel agonist. In rat ventricular myocytes, zocopride significantly enhanced I_{K1} with no effect on other ion channels, transporters, or pumps [14,15]. As a pharmacological tool, it shows antiarrhythmic effect in aconitine-toxication or acute myocardial infarction rat model.

The calcium/calmodulin-dependent protein kinase II (CaMKII) is a multi-functional serine/threonine kinase with critical roles in Ca²⁺ cycling, cell excitability, and cell function. Emergent evidence revealed the correlation of CaMKII and I_{K1} relevant for HF and arrhythmias [16,17], thus, the present study aimed to provide substantial support on the hypothesis that I_{K1} is a promising target for pharmacological controlling of lethal arrhythmias post MI via a CaMKII signaling.

2. Materials and methods

2.1. Animals and ethics

The investigation conformed to the guidelines for the Care and Use of Laboratory Animals (NIH, revised 2011) and followed the approval of the Ethics Committee of Shanxi Medical University Bioethical. Male Sprague-Dawley (SD) rats (weight 220–250 g) were provided by the Laboratory Animal Research Center of Shanxi Medical University (Taiyuan, China). The animals were housed under standard conditions: temperature 20–24 °C, humidity 40–60%, 12:12 h L: D cycles with maximal light intensity up to 200 lx. Animals were fed with standard chow and water ad libitum.

2.2. Telemetry of electrocardiogram (ECG)

2.2.1. Regular ECG telemetry

The rats were randomly divided into 4 groups, Sham, sham + Zac, MI, MI + Zac. MI was induced by ligating the left main coronary artery as previously described [14]. In brief, after sodium pentobarbital (65 mg/kg, *i.p.*) anesthesia, the rats were intubated and ventilated by an animal respirator (DH-1, Chengdu Instrument Factory, Chengdu, China) with a tidal volume of 30 mL/kg body weight and a rate of 60 tides/min. Left thoracotomy was performed in the fourth intercostal space. The proximal portion of left coronary artery was ligated by a 6–0 suture. In sham rats, the 6–0 suture was placed around the proximal portion of the left coronary artery without ligation. For telemetric ECG recording, a telemetry transmitter (Techman Soft, Chengdu, China) was secured in the abdominal cavity, and the leads were tunneled under the skin and attached to the underlying tissue. The cathode was placed in a V4–V5 position and the anode was placed under the right axilla. Zocopride at 15 μg/kg or the same volume saline was injected intraperitoneally immediately after the surgery. From the second day, all rats received either normal saline or zocopride at 15 μg/kg once a day by intraperitoneal injection. The dosage of zocopride referred to acute MI investigation [14] and pilot study as an optimal dosage. The rats were housed in individual cages placed on a receiver which continuously captured the ECG signals, independently of animal activity. The ECG signals were displayed in a real-time fashion with a computer program (BW-200, Techman Soft, Chengdu, China). ECG data were

continuously recorded for the following 4 weeks and stored for analysis.

2.2.2. Provocation test

Significant arrhythmia has been reported in patients with structural heart disease when isoproterenol (Iso) used for provocation [18]. In present study, acute injection of Iso was applied to mimic a catecholamine surge under conditions of healing MI. At the end of fourth week post MI or sham, all the radiotelemetric rats were injected with Iso (1.28 mg/kg) through tail vein. ECGs were recorded for 1 h followed provocation. Data of arrhythmias including supraventricular and ventricular arrhythmias were monitored, analyzed and compared between groups. Finally, rats were sacrificed and cardiac tissues were harvested for western blotting.

2.2.3. Evaluation of arrhythmias

Ventricular arrhythmias and supraventricular ectopic activity was evaluated according to the diagnostic criteria advocated by the Lambeth Convention [19]. ECGs were analyzed to determine the total episodes of supraventricular premature contraction (SPVC), atrioventricular conduction block (AVB), premature ventricular contraction (PVC), episode and duration of VT and VF.

2.3. Western blotting

Proteins from rat left ventricles were loaded (40 μg per lane) on 5–10% acrylamide gels. After electrophoretic transfer, the nitrocellulose membranes were incubated overnight at 4 °C with the different antibodies. Anti-mouse Kir2.1 monoclonal antibody (Sigma), anti-rabbit CaMKII and phosphorylated CaMKII (Cell Signaling) monoclonal antibodies were used at dilution 1:1000, respectively. Anti-rabbit GAPDH monoclonal antibody (1:2000, Sigma) was used as the loading control in each case. Bound primary antibody was revealed using a secondary peroxide-conjugated anti-rabbit or mouse IgG antibody (1:2000) and ECL detection according to the manufacturer's instructions. The blots were quantified by Image J.

2.4. Induction of delayed afterdepolarization (DAD) and triggered activity (TA) in rat papillary muscle

Rat ventricular papillary muscles were isolated from normal adult SD rats as previously described [20]. After anesthesia with pentobarbital sodium (65 mg/kg, *i.p.*), the heart was rapidly excised through a median sternotomy and immersed in cold (4 °C) oxygenated Tyrode's solution. The LV anterior papillary muscle about 5–6 mm long and 2–3 mm wide, was removed and pinned on silicon rubber in the center of a Perspex chamber. The papillary muscle was continuously superfused with Tyrode's solution bubbled with a mixture of 95% O₂–5% CO₂ equilibrated at 37 °C. The length of the muscle was adjusted by movement of the transducer until the resting tension was 20–40 mg. After stabilization for 1 h, the conditional stimuli, train of 10 stimuli at a basic cycle length (BCL) of 300 ms, 2-ms duration, twice threshold intensity were applied to elicit action potentials through a bipolar Ag-AgCl electrode from an electronic stimulator (SEN-3201, Nihon Kohden). The action potential were recorded with a 3 mM KCl-filled conventional microelectrode (resistance 10–20 MΩ) connected to a high input impedance amplifier (MEZ 8201, Nihon Kohden). The papillary muscles with spontaneous DAD/TA were discarded. Then Iso at 1 μmol/L and train stimuli was used to induce DAD and TA. In zocopride-pretreated group, the muscle preparation was superfused first with Tyrode's solution containing zocopride (1 μmol/L) for 10 min, then subjected to 1 μmol/L Iso. The incidence of DAD and TA (equaled to the ratio of the numbers of the preparations in which DAD and TA occurred to the total the preparation number) in the absence and presence of zocopride was measured.

2.5. Data analysis and statistics

We used two calculation methods to measure the occurrence of arrhythmias. One was the time-course analysis from the database of each rat in the same group each day until to 28th day. Another method was the average episodes of arrhythmias per rat and per day which analyzed from the accumulated data of each rat in terms of the survival days. All data were presented as mean \pm standard error (SEM), and analyzed by Least-Significant Difference (LSD) and Games-Howell tests of ANOVA (analysis of variance). The statistical difference of the occurrence frequency for an individual type of arrhythmias and mortality between groups were assessed using the χ^2 (chi square) test of two variables. Statistical differences were considered significant if the P value was < 0.05 .

3. Results

3.1. The mortality rate of post-MI rats

As shown in Supp. Table 1, MI and cardiac remodeling that followed led to cardiac death in some rats. There are 2 rats in MI group and 1 rat in MI + Zac group died within 3 h after acute MI. The mortality of MI rats post 28 days was 50%. In zacopride treated groups, the mortality declined down to 20%, but did not reach a statistical significance compared with MI group. The mortality in sham or sham + Zac group is 10%, respectively.

3.2. Zacopride suppressed ventricular arrhythmias in conscious post-MI rats

The occurrence of arrhythmias with MI was significantly higher than that in sham and zacopride treatment groups (Fig. 1). Throughout 28 days post coronary occlusion, the episodes of AVB, PVC, VT and VF showed two peaks, the first on the first day, and the second in the third and fourth week. The episodes of SPVC strikingly ascended post MI all through the 4 weeks. Zacopride treatment decreased the ventricular arrhythmias as well as AVB, but had no significant effect on SPVC.

Fig. 2 showed the average episodes of arrhythmias on 1st day, 28th day, and within 28 days in each rat post acute MI. Compared with sham rats, the total episodes of SPVC occurred from early to late phase of MI (Fig. 2A). From the time-course of SPVC within 28 days (Fig. 1), there is an upstroke in the late phase of MI, and zacopride decreases the episodes of SPVC ($P < 0.05$). But on the whole, zacopride treatment did not show significantly protection on SPVC. In MI rats, AVB and ventricular arrhythmias including PVC, VT/VF were increased all through 28 days compared with sham rats. VF was occasionally observed so not counted individually. Overall, the average episodes of AVB and PVC, the total duration of VT/VF (Fig. 2B–D, $P < 0.05$) in zacopride treated rats were markedly decreased compared with that in MI group. In the sham rats, zacopride did not exert significant effect on the ECG. These data suggested that zacopride could suppress ventricular arrhythmias, but had no significant effect on the supraventricular arrhythmias post-MI.

3.3. Zacopride suppressed acute Iso-provoked arrhythmias in conscious post-MI rats

At the endpoint of 28 days post MI, isoproterenol (1.28 mg/kg) was injected intravenously in telemetry rats before they were executed. As Fig. 3 showed, prominent ventricular arrhythmias including PVC, VT and VF occurred in the post MI rats. In detail, all rats suffered PVC (total episodes of 1073 ± 172), 80% with VT (total duration of 109.2 ± 30.0 s), and 40% with VF (21.8 ± 13.4 s). Zacopride treatment strikingly decreased the occurrence of ventricular arrhythmias, including PVC, VT and VF, upon Iso challenge in MI rats ($P < 0.01$ or $P < 0.05$). In sham rats, zacopride also prevented the episode of PVC induced by isoproterenol ($P < 0.01$).

3.4. Zacopride upregulates the expression of Kir2.1 and inhibits the activation of CaMKII in the ventricles post MI

The molecular basis of I_{K1} channel in the heart has been ascribed to members of Kir2.x sub-family comprising Kir2.1 (KCNJ2), Kir2.2 (KCNJ12) and Kir2.3 (KCNJ4) channels [13]. We have demonstrated that Kir2.1 is the predominant isoform in rat ventricles, and zacopride is exactly a Kir2.1 specific agonist [14,21]. As Fig. 4 showed, post four week-MI, the ventricle Kir2.1 expression was significantly down-regulated ($P < 0.01$), while p-CaMKII was upregulated ($P < 0.01$) compared with the sham hearts. Zacopride treatment largely restored the expression of Kir2.1 ($P < 0.01$) and p-CaMKII ($P < 0.05$).

3.5. Zacopride suppressed Iso-induced DAD and TA in adult rat papillary muscles in vitro

To clarify the potential electrophysiological mechanism of arrhythmias post MI, we recorded the transmembrane potentials in adult rat ventricular papillary muscle preparations and observed the effect of zacopride on Iso-induced DAD and TA. In the presence of 1 $\mu\text{mol/L}$ Iso, DAD or TA occurred after a train stimulus of 10 pulses at BCL of 300 ms (Fig. 5A). Zacopride at 1 $\mu\text{mol/L}$ significantly decreased the incidence of DAD and associated TA induced by Iso from 87.5% (7 of 8 preparations) to 25.0% (2 of 8 preparations) (Fig. 5B, $P < 0.05$).

4. Discussion

Up to date, the molecular mechanisms of arrhythmias are still not well known. Among various ionic channels which are involved in the formation of action potential, I_{K1} is a major determinant of resting potential and late phase repolarization of action potential, and down-regulation of I_{K1} reportedly contributed to lethal arrhythmias in the failing heart [22]. In a parallel study from our group [23], zacopride was applied in the modulation of tissue repair post MI. After 4 weeks of MI, the rat hearts underwent healing and some developed to heart failure (EF $< 50\%$). Zacopride (15 $\mu\text{g/kg/d}$) treatment could inhibit maladaptive tissue repair and improves cardiac function via activating I_{K1} . In present study, the main finding is that I_{K1} /Kir2.1 channel might be a novel target for the lethal ventricular arrhythmias via a CaMKII signaling in healing MI. Moderately enhancing I_{K1} (such as by zacopride), accordingly restoring the depolarized resting potential and shortening the prolonged the duration of action potential (APD) might be the critical electrophysiological mechanisms of antiarrhythmic strategy in cases of healing MI.

4.1. Moderately enhancing I_{K1} may suppress ventricular arrhythmias in healing MI rats via a CaMKII signaling

The hypothesis that enhancing I_{K1} counteracts MI-associated arrhythmias basically aroused from three viewpoints. First, many observations have revealed that arrhythmogenesis post MI is partly due to the decreases of resting potential and I_{K1} [24–26]. Moderate enhancing I_{K1} is a compensation for I_{K1} deficiency and restoration for resting potential to a physiological state. Second, proper enhancing I_{K1} and resultant resting potential hyperpolarization are beneficial to eliminate any abnormal automaticity. Third, I_{K1} suppression reduced the ratio of effective refractory period to APD at 90% of repolarization, thus abbreviated the postrepolarization refractoriness without marked effects on the overall APD [22]. It demonstrated how inhibition of I_{K1} may contribute to proarrhythmia in HF or Andersen syndrome. Enhancing I_{K1} may counteract this vulnerability to reentrant arrhythmia. Three viewpoints and the data from our serial studies [14,15,21] help to establish a new antiarrhythmic theory, i.e., enhancing I_{K1} , rather than decreasing I_{K1} , is a promising antiarrhythmic mechanism or strategy for lethal ventricular arrhythmias, at least in the case of healing MI.

CaMKII is enriched in hearts and can be activated by increased

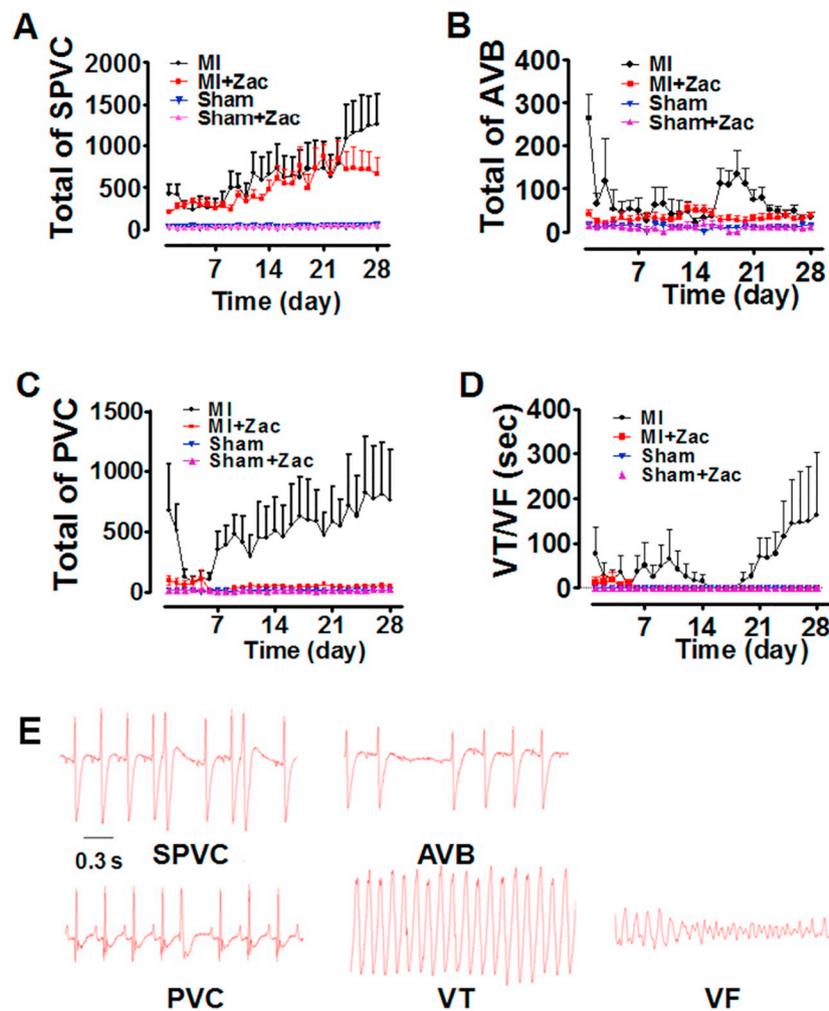


Fig. 1. (A–D) Time courses of various types of arrhythmias in conscious rats in four weeks post MI. Data were presented as mean \pm SEM. (E) Representative ECGs of supraventricular and ventricular arrhythmias recorded by telemetry in conscious post MI rats. SPVC, supraventricular premature contraction. AVB, atrioventricular conduction block. PVC, premature ventricular contraction. VT, ventricular tachycardia. VF, ventricular fibrillation.

intracellular Ca^{2+} and ROS [27]. CaMKII was shown to functionally modulate the membrane excitability of cardiomyocytes by affecting most known voltage-gated ion channels and calcium modulator, such as L-type Ca^{2+} channels, voltage-gated Na^{+} channels, multiple voltage-gated K^{+} Channels, ATP-sensitive potassium channels, chloride channels, SR Ca^{2+} release ryanodine receptor (RyR2) channels and phospholamban (PLN) [16,17]. As a key modulator for membrane resting potential and excitability, I_{K1} is also regulated by CaMKII. Chronic CaMKII over-expression could downregulate I_{K1} channels [28], whereas inhibition of CaMKII increases I_{K1} channel density which partially accounted for the shortening of APD [29]. These findings agree well with the observations in the present study. Approaches of targeting I_{K1} might bring antiarrhythmic benefit via a CaMKII signaling.

4.2. Moderately enhancing I_{K1} is beneficial to improve electrical remodeling in healing MI rats

In the present study, long-term ECG telemetry MI model, rats displayed SPVC AVB, and ventricular arrhythmias. Zacopride decreased the AVB but had no significant effect on the SPVC. These results inspired us that I_{K1} agonist, especially the Kir2.1 agonist such as zacopride, might be a novel therapeutic target for ventricular arrhythmias.

In the process of repairing post MI, changes in ion channel expression and function, for instance, altered $I_{\text{Ca-L}}$, reduced I_{to} , I_{K1} , I_{Kr} and I_{Ks} , enhanced Na^{+} - Ca^{2+} exchanger (NCX) and downregulated Na^{+} - K^{+}

pump, may occur either in the peri-infarct area or in the remote regions of the infarct scar [26,30,31]. These changes are known as electrical remodeling. Reduction of I_{K1} and prolongation of APD are hallmarks of electrical remodeling post MI [23–26]. I_{K1} reduction depolarizes the resting potential and increases the diastolic membrane resistance. The latter resultantly amplifies the fluctuation of membrane potential, and makes higher chance of DAD to reach the threshold for evoking a premature action potential [32]. The reduction of I_{K1} (alone or together with I_{Kr} and I_{to}) also decreases the repolarizing reserve of K^{+} currents, thereafter postpones the late phase repolarization and increases the vulnerable window for reactivation of voltage-gated Ca^{2+} channels. Both APD prolongation and Ca^{2+} influx markedly increase the risk for EAD. Zacopride via enhancing I_{K1} could reverse the depolarization and instability of resting potential, therefore decrease cardiac excitability and autorhythmicity. In addition, enhancing the outward component of I_{K1} may markedly shorten the final phase of action potential repolarization thereby is beneficial to diminish the EAD, DAD and triggered activity.

In cardiac tissue, intrinsic APD dispersion varies across several axes including transmural, left-right and apico-basal axes [33,34], and the APD dispersion can be strengthened by ischemia or chronic MI [35,36]. Increased dispersion of action potential repolarization exists among the infarct area, the border zone and the remote viable myocardium [37]. APD is much longer in the epicardium than in the endocardium remote from the infarct zone, [26]. All these alterations facilitate the risk of re-

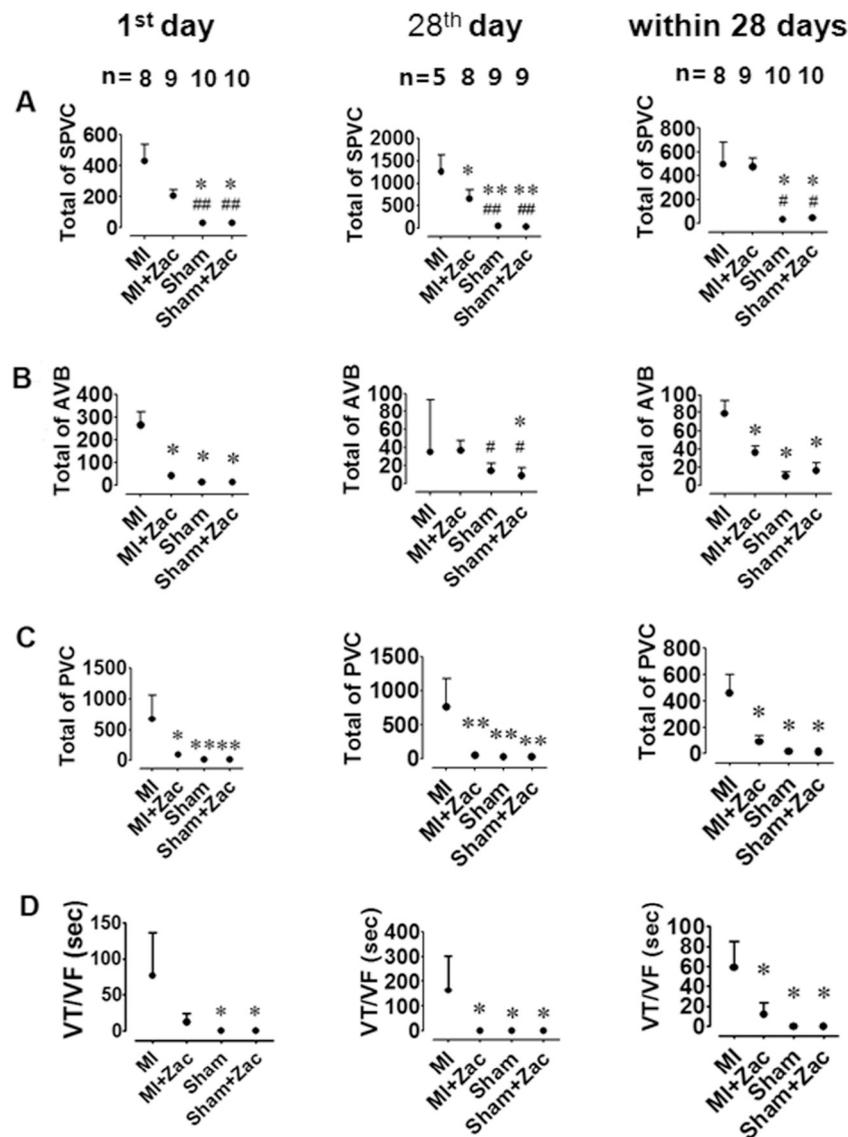


Fig. 2. Statistical comparison of the arrhythmias in conscious rats from different groups. Average episodes of a certain type of arrhythmia on 1st day, 28th day, and within 28 days after surgery in each rat were calculated (mean \pm SEM). (A) Total episodes of SPVC. (B) Total episodes of AVB. (C) Total episodes of PVC. (D) Total episode duration of VT/VF. SPVC, supraventricular premature contraction. PVC, premature ventricular contraction. VT, ventricular tachycardia. VF, ventricular fibrillation. * $P < 0.05$, ** $P < 0.01$ vs. MI. # $P < .05$, ## $P < 0.01$ vs. MI + zac.

entrant arrhythmias [38,39]. By shortening the APD, zacopride might resultantly diminish the APD dispersion. More importantly, via hyperpolarizing the resting potential, zacopride may simultaneously increase the voltage-dependent Na^+ channel availability, accelerate the conduction velocity of myocardium in the border zone, and thus may benefit the prevention or termination of reentrant activity.

4.3. Moderately enhancing I_{K1} improves the tolerance of heart to calcium overload and ischemia post MI

Many life stressors, such as high-intensity exercise, anger, hostility and emergency, have been confirmed to overactivate the sympathoadrenal system and trigger arrhythmias [2–5]. In some cardiovascular events, sympathetic provocation is the dominant factor for the high risk of malignant arrhythmogenesis [40]. Isoproterenol is a non-specific β -adrenergic agonist and applied in present study to mimic stress-induced sympathoadrenal activation and elicit intracellular calcium overload. Intrinsic catecholamine and ischemia in healing MI are sufficient to enhance intracellular Ca^{2+} loading and hamper the buffering

capacity of hearts to acute stress. Long-term application of zacopride could enhance the tolerance of hearts to Ca^{2+} overload and ischemia, resultantly reduce the generation of DADs and triggered arrhythmias. The convincing data demonstrated in present study and our previous work [14,15,41] suggests that improvement of calcium handling or energy metabolism secondary to I_{K1} activation might be a novel anti-arrhythmic mechanism.

In summary, zacopride suppresses lethal ventricular arrhythmias post-MI via directly enhancing $I_{K1}/\text{Kir}2.1$. Zacopride and the potential analogues which have similar action of zacopride may be developed to prevent or treat lethal ventricular arrhythmias in cases of acute MI and healing MI.

5. Limitation

We found that zacopride improved the AVB post MI, but did not investigate the mechanism underlying this action. AVB is a common complication of MI [42] and can be induced by any disturbances of electrical conduction within the atrioventricular (AV) node, the bundle

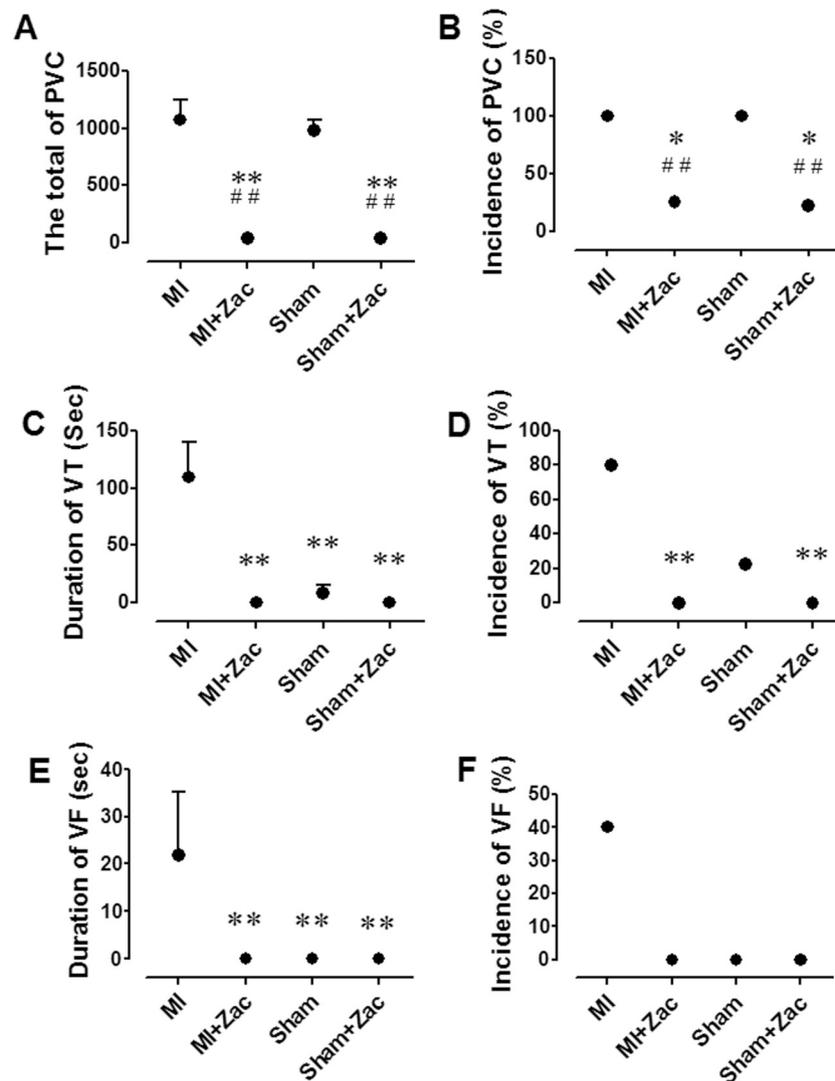


Fig. 3. Effects of zacopride on acute I_{50} -provoked arrhythmias in PMI telemetry rats. (A) The total of PVC, as the total number of episodes in 1 h. (B) The incidence of PVC. (C) The duration of VT: the total episode duration of VT in 1 h. (D) The incidence of VT. (E) The duration of VF: the total episode duration of VF in 1 h. (F) The incidence of VF. MI, myocardial infarction. Zac: zacopride; Iso, isoproterenol. PVC, premature ventricular contraction. VT, ventricular tachycardia. VF, ventricular fibrillation. N = 5, 8, 9, 9 in MI, MI + zac, sham, sham + zac, respectively. * $P < 0.05$, ** $P < 0.01$, vs. MI; ## $P < 0.01$ vs. sham.

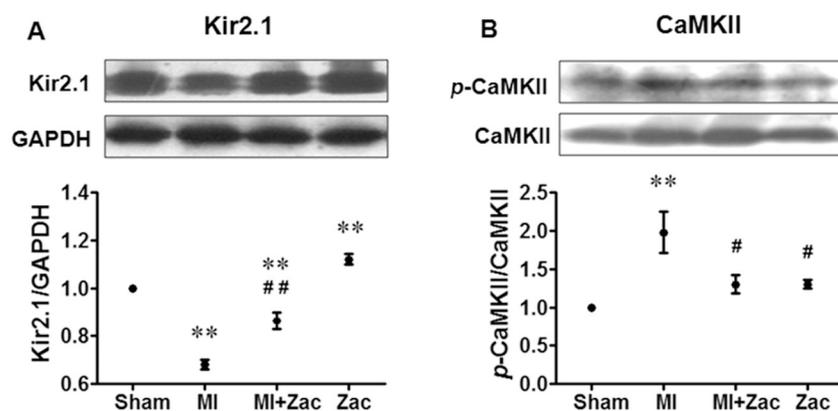


Fig. 4. Western blotting showing the expression of Kir2.1 and CaMKII in the myocardium of rats from different groups. (A) Kir2.1 expression relative to GAPDH on the 28th day after surgery in different groups. Note that zacopride (Zac) restored the downregulated Kir2.1 expression in post-MI rats. (B) p-CaMKII expression relative to total CaMKII on the 28th day after surgery in different groups. CaMKII was hyperactivated post MI and largely restored by zacopride treatment. N = 3. * $P < 0.05$, ** $P < 0.01$, vs. sham. ## $P < 0.01$ vs. MI.

of His, and the bundle branches. According to Hibino et al. [13], little I_{K1} was expressed in sinoatrial (SA) and nodes, but rich in atrial and ventricular myocytes, His bundle and Purkinje fibers. I_{K1} and resting potential were reportedly decreased in canine subendocardial Purkinje myocytes from the 48-hour infarcted heart [43]. The decremental

Kir2.1 expression in Purkinje fibers as well as slowed His-Purkinje conduction was demonstrated in the chronic HF dogs [44]. The decrease of I_{K1} /Kir2.1 prolongs the refractoriness of the atrial and ventricular muscle as well as the His-Purkinje bundle, resultantly underlying the pathogenesis of AVB. As an I_{K1} /Kir2.1 agonist, zacopride is

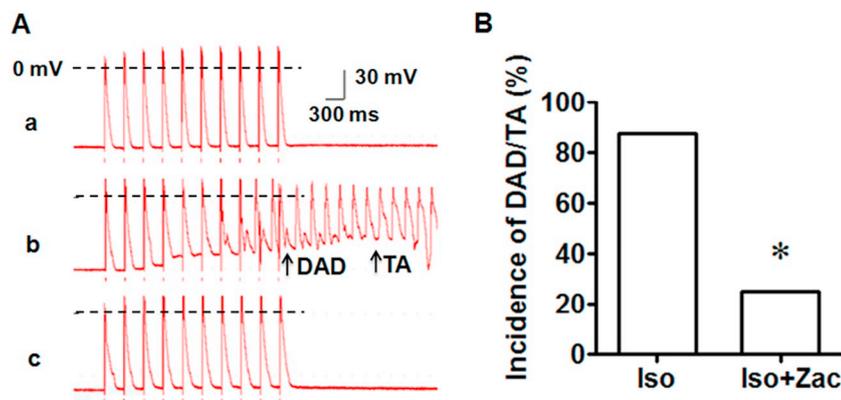


Fig. 5. Zacopride pretreatment inhibited Iso-induced delayed afterdepolarization (DAD) and triggered activity (TA) in rat papillary muscles. (A) Onset of the train of 10 stimuli, (a) control, none DAD occurred; (b) Iso (1 $\mu\text{mol/L}$), DAD and TA occurred; (c) zacopride (1 $\mu\text{mol/L}$) + Iso (1 $\mu\text{mol/L}$), no visible DAD and TA occurred. (B) Zacopride significantly decreased the incidence of Iso-induced DAD and TA. $N = 8$. * $P < 0.05$ vs. Iso.

theoretically and practically effective against MI-induced AVB. In addition, via hyperpolarizing the RP, zacopride may increase the voltage-dependent Na^+ channel availability, accelerate the conduction velocity of atrial and His-Purkinje bundle, in turn counteract AVB. However, the precise mechanism merits further investigation.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lfs.2019.117075>.

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Author contributions

X.W. Z., L. Z. and X. Q. performed experiments, analyzed data. L.J. Z. performed experiments. D.M. W. analyzed data. Q.L. F. contributed to experimental design. Q.H. L. designed the experiments and drafted the manuscript. J.M. C. and B.W. W. designed the experiments and revised the manuscript. All authors approved final version of manuscript.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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