Original Article

Effects of ramipril on ventricular arrhythmia after myocardial infarction in rabbits

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BACKGROUND: Ventricular arrhythmia (VA) is one of the most common complications of myocardial infarction (MI), and ventricular tachycardia and fibrillation are the main causes for sudden cardiac death. This study aimed to explore the effect of ramipril on the occurrence of VA and its mechanism after MI in rabbits.

METHODS: Twenty-four New Zealand rabbits purchased from the Wuhan Laboratory Animal Research Center were divided into three groups: sham-operated (SHAM) group ($n=8$), MI group ($n=8$) and MI with ramipril (RAM) group ($n=8$). Rabbits in the SHAM group received a median sternotomy without ligation of the left ventricular coronary artery. Rabbits in the MI and RAM groups received a median sternotomy followed by ligation of the left coronary artery. The successful anterior MI was confirmed by elevation of the ST segment with more than 0.2 mV in lead II and III. After MI, rabbits in the RAM group were fed with intragastric ramipril (1 mg/kg per day) for 12 weeks. Before and 12 weeks after MI in the three groups, ventricular tachycardia or fibrillation (VT/VF) episodes and MAP in cardiocytes of the epicardium, mid-myocardium and endocardium were recorded by a multichannel physiograph. Student's t test and ANOVA were used for statistical analysis.

RESULTS: VT/VF episodes were decreased more markedly in the RAM group than in the MI group after 12 weeks (2.6 ± 0.8 vs. 12.4 ± 2.9 , $P < 0.05$). Twelve weeks after MI, the duration of repolarization for 90% (APD_{90}) of three-tier ventricular myocytes in the MI group was longer than that before MI (258.2 ± 21.1 vs. 230.1 ± 23.2 , 278.0 ± 23.8 vs. 245.8 ± 25.4 , 242.6 ± 22.7 vs. 227.0 ± 21.7 , $P < 0.05$). However, the APD_{90} was not significantly different at 12 weeks before and after MI in the RAM group ($P > 0.05$). Moreover, the transmural dispersion of repolarization (TDR) was increased more markedly 12 weeks after MI in the MI group than in the SHAM and RAM groups (36.2 ± 10.2 vs. 18.7 ± 6.2 , 24.9 ± 8.7 , $P < 0.05$). But the TDR was not significantly different between the RAM and SHAM groups (18.7 ± 6.2 vs. 24.9 ± 8.7 , $P > 0.05$).

CONCLUSION: Ramipril may reduce the incidence of malignant ventricular arrhythmia via improvement of transmembrane repolarization heterogeneity after MI.

KEY WORDS: Myocardial infarction; Ventricular arrhythmia; Monophasic action potential duration; Transmural dispersion of repolarization; Ramipril; Rabbits

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INTRODUCTION

Ventricular arrhythmia (VA) is one of the most common complications of myocardial infarction (MI), and ventricular tachycardia and fibrillation are the main causes for sudden cardiac death.^[1] The role of ventricular remodeling in long-term survivors after MI has been fully recognized,^[2] and clinical and experimental data have definitely suggested that the risk of VA occurrence

is correlated with the degree and characteristics of ventricular remodeling after MI.^[3,4] The most common electrophysiological abnormality of myocardial hypertrophy presented an extended duration of action potential,^[5] and the abnormal change of ion current in ion channels of hypertrophic cardiac myocytes and the so-induced membrane potential change serve as the electrophysiological foundations of VA. The extended

duration of action potential is likely to cause dispersed repolarization and after-depolarization, which in turn induce various arrhythmias.^[6] Therefore, a further study on the mechanism of VA after MI is of great significance in preventing and treating VA to reduce the incidence of sudden cardiac death due to coronary heart disease.

The abnormal electrophysiological change of hypertrophic myocardial cells in non-MI regions plays a critical role in the occurrence of ventricular arrhythmias after MI.^[7] After MI, AngII produced in circular and local tissues promotes myocardial remodeling. The replacement of part of myocardial tissues by fibrous tissues promotes the anisotropy of reentry, which easily causes reentrant arrhythmias.^[8,9] Clinically, it was also confirmed that angiotensin-converting enzyme inhibitors (ACEI) could effectively inhibit the fibrosis of the left ventricular myocardium.^[10] Based on the above finding, the present study aimed to explore the effects of ACEI ramipril on the monophasic action of the left ventricular myocardium in an attempt to explore the possible mechanisms of ventricular arrhythmias.

METHODS

Animal preparation

The rabbit model of MI used in this study was previously described.^[11] Twenty-four rabbits, weighing 1.5–2.0 kg, were randomly divided into three groups: sham operation group (SHAM, $n=8$), myocardial infarction group (MI, $n=8$), and ramipril (RAM, $n=8$) group. Under sterile conditions, the rabbits were anesthetized with 3% pentobarbital solution given intraperitoneally. Thoracotomy was performed through a left parasternal incision, while the pericardium was incised and the anterior wall of the left ventricle exposed. The left anterior descending (LAD) coronary arteries of the rabbits in the MI and RAM groups were identified and carefully separated, and then they were ligated downward 3 mm, from where the first diagonal artery branches out. The rabbits in the SHAM group underwent thoracotomy without ligation of the LAD coronary arteries. Postoperatively, each rabbit received 400 000 IU penicillin intramuscularly twice daily for 2 days, and they were fed from the second day for 12 weeks after surgery. The rabbits in the RAM group were administered with 1 mg/kg of ramipril every day. ECG limb leads II and III were observed, and Q wave in leads or ST segment elevation by above 0.2 mV demonstrated the formation of MI.

Electrophysiological detection

Electrodes recorded by monophasic action potential (MAP) were designed according to the report.^[12] MAPs of the epicardium, midmyocardium and endocardium at MI

area in the left ventricle were recorded. In order to guarantee two different recordings at the same point, the distal point at 10 mm to the first diagonal branch of the LAD artery was taken as the reference point, and the point at 3 mm to its right left as the recording point. Electrodes were connected with a polygraph system (LEAD 2000B), and waveforms were stored in hard disc of the computer for review and analysis. Whether recording electrodes reached the epicardium, midmyocardium and endocardium of the left ventricular anterior wall was mainly judged according to the depth of penetration. Generally, the thickness of the left ventricular anterior wall in rabbits was 4 mm. The vertical spaces of the electrode tips in the epicardium, midmyocardium, and endocardium were 2 mm, and when the epicardium electrode contacted with the epicardium, other two electrodes reached their corresponding cellular layers. APD₉₀ was defined as the duration from the beginning of MAP to 90% repolarization, and TDR as the difference between the longest and the shortest APD₉₀ of the three layers at the same point. The limb lead surface electrocardiograms were monitored during the experiment.

The epicardium, midmyocardium and endocardium at the intended ligated site before MI and those at MI area in the left ventricle 12 weeks after MI were respectively given procedure stimulations twice, with a stimulus intensity of two times the pacing threshold during the diastolic phase, S1S1=300 ms, S1S2=250 ms, in –10 ms decrements to S1S2=50 ms. Inductions of VA after the first open heart surgery and 12 weeks after MI in each group were respectively observed.

Statistical analysis

Data were presented as mean±SD and analyzed by SPSS10.0 statistical software. Student's *t* test and variance analysis were carried out. $P<0.05$ was considered statistically significant.

RESULTS

Induced arrhythmia before and after MI

Programmed stimulation didn't induce arrhythmia in the MI or RAM group before MI, or in the SHAM group. In 12 weeks after MI, programmed stimulation induced arrhythmia in the MI group, including monomorphic and polymorphic ventricular tachycardia, torsades de pointes, ventricular fibrillation, etc, and the episode of arrhythmia was 12.4 ± 2.9 . In the RAM group, arrhythmia took the forms of monomorphic and polymorphic ventricular tachycardias, and the episode was 2.6 ± 0.8 . Compared with the MI group, the RAM group had a much lower episode of induced arrhythmia ($P<0.05$) (Figure 1).

Figure 1. Torsades de pointes induced by programmable stimulation in the MI group. From the top to bottom, induced torsades de pointes when MAPs of the epicardium, midmyocardium and endocardium were recorded in ECG lead II respectively.

Table 1. Comparison of APD₉₀ of the epicardium, midmyocardium and endocardium before MI and 12 weeks after MI in the three groups (*n*=8)

Groups	Before MI	12 weeks later after MI
SHAM		
Endocardium	230.1±23.1	232.2±23.5
Midmyocardium	244.3±24.1	243.7±24.3
Epicardium	225.4±22.6	225.0±21.6
MI		
Endocardium	230.1±23.2	258.2±21.1*
Midmyocardium	245.8±25.4	278.0±23.8*
Epicardium	227.0±21.7	242.6±22.7*
RAM		
Endocardium	235.3±21.2	236.5±22.9
Midmyocardium	246.0±23.6	259.4±26.6
Epicardium	229.6±21.9	234.1±22.8

Comparisons of APD₉₀ of the same myocardium in the same group before and after MI, **P*<0.05.

Table 2. Comparison of TDR among the three groups before MI and 12 weeks after MI (*n*=8)

Groups	Before MI	12 weeks after MI
SHAM	19.0±6.6	18.7±6.2
MI	18.6±5.4	36.2±10.2*
RAM	17.0±6.1	24.9±8.7

Compared to the SHAM or RAM group, **P*<0.05.

Effect of ramipril on APD₉₀ at MI area

Before MI, no significant difference was found in APD₉₀ of the endocardium, midmyocardium and epicardium in the different groups (*P*>0.05). At 12 weeks after MI, the APD₉₀ values of three layers of ventricular myocardia in the MI group were longer than those before MI (258.2±21.1 vs. 230.1±23.2, 278.0±23.8 vs. 245.8±25.4, 242.6±22.7 vs. 227.0±21.7, *P*<0.05), and the APD₉₀ of the midmyocardium was increased more markedly, but no statistical significance difference was seen in the three layers before and after MI. In the RAM group, there was no significant difference in APD₉₀ of any layer before and after MI (236.5±22.9 vs. 235.3±21.2, 259.4±26.6 vs. 246.0±23.6, 234.1±22.8 vs. 229.6±21.9, *P*>0.05). In the SHAM group, also no significant difference was found (*P*>0.05) (Table 1).

Before MI, no significant difference was noted in TDR among the three groups. At 12 weeks after MI,

TDR in the MI group was more significant than that of the SHAM or RAM group (36.2±10.2, 18.7±6.2 and 24.9±8.7, respectively; *P*<0.05); but there was no significant difference between the RAM and SHAM groups (*P*>0.05) (Table 2).

DISCUSSION

MAP demonstrates as the local electro-activity of myocardiac cell mass in extracellular recordings, and its waveforms can truly reflect the morphology and phases of transmembrane action potentials, especially the phases of depolarization. In recent years, MAP has been widely used in studies on the mechanism of arrhythmic occurrence, the evaluations of anti-arrhythmic drugs, etc. In this study, different from the method adopted in previous studies of multipoint recordings for MAPs by using a single electrode,^[13] a self-designed combination electrode was used to synchronously record MAPs of three layers of the left ventricular myocardium. Both methods produced the same results. In addition, compared with the conventional method, the method used in this study has its own advantages. It could obtain stable graphs, which objectively reflected the repolarization differences among the three layers of the myocardium, and it could decrease the damages caused by a single electrode for multipoint recordings and the errors due to non-synchronous recordings. Thus, this method may be reliable for further studies on MAP.

After MI, electrical remodeling and tissue remodeling occur in the myocardium. This is very likely to induce malignant ventricular arrhythmia^[14] and increase the mortality. Though ACEI can reverse myocardial hypertrophy, which has been confirmed both experimentally and clinically, rare reports about their effect on electrophysiology after MI have been reported. Therefore, in this study we investigated the effect of ramipril on the APD₉₀ of the three layers of the myocardium after MI.

Ventricular electrophysiological heterogeneity comes from both cell factors and tissue factors. A lot of experiments (*in vitro* and *in vivo*) in recent years have proved that at least three types of myocytes with different electrophysiological characteristics exist in the ventricular wall in dogs, rabbits and humans, i.e. endocardial, midmyocardial and epicardial cells. The increase of transmembrane repolarization heterogeneity of the myocardium is one of the electrophysiological mechanisms of malignant ventricular arrhythmias. In pathologic states such as cardiac failure, myocardial infarction, myocardial hypertrophy, and cardiomyopathy, the electrophysiological heterogeneity as the main cause

of ventricular arrhythmias was increased.^[13,15,16]

And under normal physiological conditions, the inherent electrophysiological manifestations in three different types of myocardial cells were restrained to make the transmembrane electrophysiological heterogeneity less obvious because of the regulation of neurohumor factors and the electrotonus action between different myocardia on myocardial cells.^[17] This might explain that there were no significant differences in APD₉₀ among the three myocardial layers before MI in our study. Our results exhibited that APD₉₀ of the midmyocardium 12 weeks after MI was prolonged compared with that before MI. Though there was no significant difference between them, this prolongation increased the repolarization heterogeneity among three myocardial layers, consequently significantly increased TDR compared with that before MI. In addition, procedure stimulation didn't induce ventricular arrhythmias in any group before MI in our study, but the stimulation did cause a variety of arrhythmias in the MI group 12 weeks after MI such as monomorphic and polymorphic ventricular tachycardia, torsades de pointes, ventricular fibrillation, etc, indicating that the increase of transmembrane repolarization dispersion after MI is the major cause of malignant arrhythmias even after the healing of MI.

Furthermore, after MI was treated with ramipril for 12 weeks in our study, the episode of procedure stimulation-induced malignant ventricular arrhythmia was significantly decreased. And TDR was restored in the RAM group, very close to that in the SHAM group and much shorter than that in the MI group. This indicated that ramipril may reduce the incidence of malignant ventricular arrhythmia via this improvement of transmembrane repolarization heterogeneity.

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Ethical approval: The local ethical committee of Wuhan Laboratory Animal Research Center approved this study (Approval No: 42007865008763).

Conflicts of interest: None declared.

Contributors: Zhong Y proposed the study, analyzed the data and wrote the first draft. All authors contributed to the design and interpretation of the study and to further drafts.

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