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Full Length Research Paper

Effect of valsartan on action potential and potassium efflux in rabbits with myocardial infarction

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The aim of this study was to characterize the effects of valsartan on ventricular tachycardia or fibrillation (VT/VF) after myocardial infarction (MI). Rabbits in the MI and valsartan (VAL) groups were subjected to median sternotomy followed by left coronary artery ligation. MI was induced among the rabbits and the VAL rabbits were given VAL orally for 12 weeks. VT/VF episodes, as well as monophasic action potentials (MAPs) were recorded. Transient potassium efflux from the myocytes isolated from the border zone of the infarcts in the left ventricular wall was recorded. In the MI group, the action potential at 90% repolarization (APD_{90}) in the ventricular myocytes was prolonged after MI. However, in the VAL group, no significant difference was observed between the APD_{90} before MI and at 12 weeks after VAL treatment. The transmural dispersion of repolarization (TDR) was increased after MI. The increase in TDR was reversed by VAL. The density transient outward potassium current (Ito) was lower in the MI group than in the sham-operated (SHAM) and VAL groups, respectively. However, no significant difference was observed between the SHAM group and the VAL group. VAL reduces electrophysiologic heterogeneity. VAL reverses the abnormal ionic flux during the plateau of the action potential.

Key words: Myocardial infarction (MI), monophasic action potential duration, transient outward potassium current, patch clamp, valsartan (VAL).

INTRODUCTION

The epicardium, mid-myocardium, and endocardium cells in the heart of animals and humans have different electrophysiologic characteristics. This electrophysiologic heterogeneity can lead to ventricular transmural reentrance and reentrant arrhythmia. Increased dispersion of ventricular transmural repolarization is one of the electrophysiologic mechanisms for malignant ventricular arrhythmia. Malignant ventricular arrhythmia is one of the critical causes of sudden cardiac death in patients with myocardial infarction (MI) (Antzelevitch et al., 1991; Wolk et al., 1999). The importance of ventricular remodeling among patients that survive MI is well known. The most important electrophysiologic abnormality of hypertrophic cardiomyocytes is prolonged action potential duration (APD) (Myerburg et al., 1992). The abnormal change in ion channel current and the membrane potential in hypertrophic cardiomyocytes are the electrophysiologic basis for arrhythmias. Prolonged repolarization produces dispersion of repolarization and after-depolarization, which then promotes different arrhythmias (Thollon et al., 1989). Therefore, developing a new strategy for preventing cardiac death and malignant ventricular arrhythmia is of considerable importance.

In recent times, the treatment of ventricular arrhythmia has remained difficult. The final prognosis among patients administered with this kind drug is unobtainable and it increases the number of sudden deaths or the total mortality. Several large-scale clinical tests have revealed that amiodarone and β-receptor blockers significantly diminish the risk of sudden death in patients with MI and heart failure. Unfortunately, their side effects limit their clinical application.

Angiotensin II (AngII) is the main active peptide in the rennin-angiotensin system. AngII is a more important peptide in the regulation of the physiologic functions of

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the cardiovascular system. However, AngII is a major arrhythmogenic peptide (de Langen et al.,1989). AngII appears to contribute to the genesis of arrhythmias through several mechanisms: direct positive inotropic effect, decreased ion channel and gap junction efficiency, decreased intercellular electrical impedance, shortening of the refractory period, excitation of autonomic nervous system, and decreased myoarchitectonic efficiency (Reiss et al., 1993; De Mello et al., 1996; Timmermans et al., 1993; Gunasegaram et al., 1999; Tsutsumi et al., 1999; Oz et al., 2005). Most of its arrhythmogenic effects are mediated through AT_1R. AngII antagonists include angiotensin-converting enzyme inhibitors (ACEIs) and AngII receptor blockers (ARBs). The antiarrhythmic properties of ACEIs and ARBs have been demonstrated in some studies. AngII and its antagonists should be studied further to determine their roles in causing arrhythmia.

Various arrhythmias follow MI, particularly, malignant ventricular arrhythmia. Malignant ventricular arrhythmias are the main cause for sudden cardiac death after MI (Aimond et al., 1999). Recent studies have shown that AngII receptor blockers (ARBs) prevent myocardial fibrosis and diastolic dysfunction (Brilla et al., 1997; Wake et al., 2005). The exact mechanism of the protective effect of ARB remains unclear. To investigate the effects of ARB on the electrophysiologic heterogeneity, the changes in the monophasic action potential (MAP) and transient potassium efflux in ventricular myocytes after MI were investigated and whether valsartan (VAL), an ARB, reverses the electrical remodeling, was evaluated.

MATERIALS AND METHODS

MI model

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Zhongnan Hospital of Wuhan University (Permit Number: 20060828001). The rabbit model of MI used in this study has been previously described (Yuan et al., 1999). Twenty-four rabbits (1.5 to 2.0 kg) were anesthetized with pentobarbital (20 mg/kg, intraperitoneal injection). The rabbits were randomly divided into three groups: the sham-operated (SHAM) group (n = 8), the MI group (n = 8), and the VAL group (n = 8). The left anterior descending (LAD) coronary artery in the MI and VAL groups was ligated 3 mm distal of the bifurcation of the first diagonal artery. The animals in SHAM group underwent thoracotomy without ligation of the LAD. After MI, the rabbits in the VAL group were given VAL orally (10 mg/kg/day) for 12 weeks.

Electrophysiological study

The electrode used to record the MAP was made as previously described (Li et al., 2003). MAP was determined using a LEAD-2000B instrument (Sichuan, China). The depth of penetration was used to judge whether the recording electrodes have reached the inner layer, the intercellular layer, or the outer layer of the anterior wall of left ventricle. The thickness of the anterior wall of the left ventricle is commonly 4 mm in rabbits, the perpendicular distance of the inner, middle, and outer electrode leads were 2 mm; when the epicardial lead reached the outer layer, the other two leads, separately reached the corresponding layers. Action potential at 90% repolarization (APD_{90}) is defined as the time between APD initiation and 90% repolarization. The transmural dispersion of repolarization (TDR) is defined as the difference between the longest and the shortest APD_{90} in the three-tier left ventricular myocardium. The electrocardiography (ECG) (II, avF) was continuously monitored. The TDR was calculated as follows: TDR = APD_{90} max - APD_{90} min.

The outer, middle, and inner layers were stimulated 20 times before and after MI; the stimulus intensity was twice the diastolic pacing threshold, S1S1 = 300 ms, S1S2 = 250 ms, in 10 ms decrements to S1S2 = 50 ms.

Isolation of myocytes

The ventricular myocytes were isolated as previously described (Xu et al., 2001). Briefly, hearts were removed from the rabbits under anesthesia, and perfused on a Langendorff apparatus at 37°C by pumping with Ca^{2+}-free Tyrode's solution. Following a 3 min perfusion with calcium-free Tyrode's solution, low calcium (100 µmol/L) Tyrode's solution containing 0.40 mg/ml type 1 collagenase was perfused for about 5 to 7 min. The heart was then detached from the cannula. The tissues of the left ventricle were cut into small pieces and placed into a beaker containing 1.5 ml of recirculated enzyme solution and 10 ml of calcium-free Tyrode's solution with 1% bovine serum albumin. These pieces were then agitated and incubated in the same solution at 37 C. After 10 min, the cell suspension was filtered. Finally, the cells were stored at room temperature for at least 1 h before use.

Electrical recording techniques

A small aliquot of the solution containing the isolated cells was placed in an open perfusion chamber (1.5 ml) mounted on the stage of an inverted microscope (Olympus, Japan). The myocytes were allowed to adhere onto the bottom of the pool for 5 to 10 min and were then superfused at 2 to 3 ml/min with the bath solution. Only rod-shaped cells with clear striations were used for the experiment. The pool was perfused with extracellular solution. Microelectrodes were pulled with a microelectrode puller (Narishige, Japan) and had a resistance of 5 to 8 MΩ when filled with electrode internal solution. After the giga-seal was formed and the membrane was ruptured, currents were recorded in voltage-clamp mode using a patch clamp amplifier (EPC-9, Germany). Capacitive-transients and series resistance were compensated. Experimental protocols, data acquisition, and storage were accomplished with pClamp 8.0 (HEKA, Germany) running on a personal computer. All experiments were conducted at 20 to 23°C.

To record transient outward potassium current (Ito), the cells were depolarized from a holding potential of -80 mV to 300 ms with different test potentials starting at 10 mV, and increased from -40 to +50 mV.

Statistical analysis

Data are expressed as mean ± standard deviation (SD) and the analysis using analysis of variance (ANOVA) and t-test were performed with Statistical Package for Social Sciences (SPSS) 10.0 software. $P < 0.05$ was considered significant. Ion current was expressed in ratio of electric current and cell surface capacitive (pA/pF) under the established voltage.

Table 1. Changes of APD_{90} before MI and at 12 weeks after, among the three groups.

Group (ms)		Pre-MI	After MI 12 weeks
SHAM	Endo	230.1 ± 23.1	232.2 ± 23.5
	Mid	244.3 ± 24.1	243.7 ± 24.3
	Epi	225.4 ± 22.6	225.0 ± 21.6
MI	Endo	230.1 ± 23.2	258.2 ± 21.1[b]
	Mid	245.8 ± 25.4	278.0 ± 23.8[b]
	Epi	227.0 ± 21.7	242.6 ± 22.7[b]
VAL	Endo	231.3 ± 23.2	237.4 ± 21.9
	Mid	245.0 ± 26.2	256.8 ± 23.6
	Epi	227.6 ± 22.5	232.5 ± 22.4

APD_{90} values (ms) in epicardium (Epi), midmyocardium (M), and endocardium (Endo) before and after MI 12 weeks; n = 8; Mean ± SD. [b]P < 0.05 versus Pre-MI.

Table 2. Changes in TDR before MI and at 12 weeks after, among the three groups.

Group (ms)	Pre-MI	After MI 12 weeks
SHAM	19.0±6.6	18.7±6.2
MI	18.6±5.4	36.2±10.2[b]
VAL	18.0±5.7	23.9±9.1

TDR in three rabbit groups before and after MI 12 weeks. n = 8; Mean ± SD; [b]P < 0.05 versus SHAM and VAL groups.

RESULTS

Changes of APD_{90}

At 12 weeks after MI, the APD_{90} in epicardium, mid-myocardium, and endocardium were obviously increased (P < 0.05) when compared with that before MI, especially in the mid-myocardium in the MI group. However, no significant difference was observed in the VAL group. APD_{90} significantly did not change before MI and at 12 weeks after in the SHAM group (Table 1).

The APD_{90} of the mid-myocardium in the SHAM group was the longest and that in the epicardium was the shortest. The changes in TDR in the MI and VAL groups were the same as that in the SHAM group after MI: APD_{90} Mid > Endo > Epi. The changes in TDR before MI and at 12 week after the three groups are shown in Table 2. The TDRs in the three groups were not significantly different before MI. At 12 weeks after MI, the TDR in the MI group was significant longer than those in the SHAM and the VAL groups (36.2 ± 10.2 ms versus 18.7 ± 6.2 ms, 23.9 ± 9.1 ms, P < 0.05). There was no significant difference between the VAL and SHAM groups (P > 0.05).

Ventricular fibrillation/tachycardia (VF/VT) events

The stimulation procedure did not induce ventricular arrhythmia before MI in the SHAM, MI, or VAL group. At 12 weeks after MI, the 20 stimulations induced arrhythmia 11.7 ± 1.8 times in the MI group, including monomorphic ventricular tachycardia (VT), multiform ventricular tachycardia, torsades de pointes, and ventricular fibrillation (VF). After a 12 week-VAL treatment, the procedure only induced monomorphic VT and multiform VT 3.2 ± 0.6 times (Figure 1).

Transient outward potassium current (Ito)

The currents were recorded 10 min after rupture of the membrane. To eliminate the influence of the operation on current, normal rabbits were observed for comparison. The results reveal that the current and current-voltage (I-V) curve of Ito in the SHAM group were not significantly different (P > 0.05). The current in the SHAM group was used as the control group. Ito was activated, because the -30 mV increased with increasing membrane potential depolarization. When the assigned voltage reached +60 mV, the current density of Ito in the SHAM group, the MI group and the VAL group were 8.56 ± 0.72, 4.51 ± 0.48, and 7.24 ± 0.68 pA/pF, respectively. The Ito current in the MI group was significantly lower than those in the SHAM group and the VAL group (P < 0.05), and there was no statistically significant difference between the VAL group and the SHAM group (P<0.05). The mean current densities under various peaks, which correspond

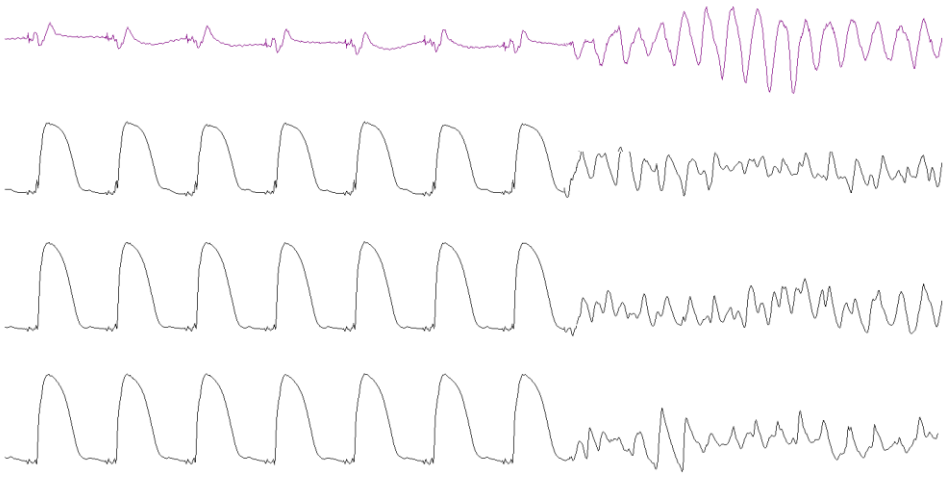

Figure 1. Graph of torsades de pointes induced by the stimulation protocol in the MI group.

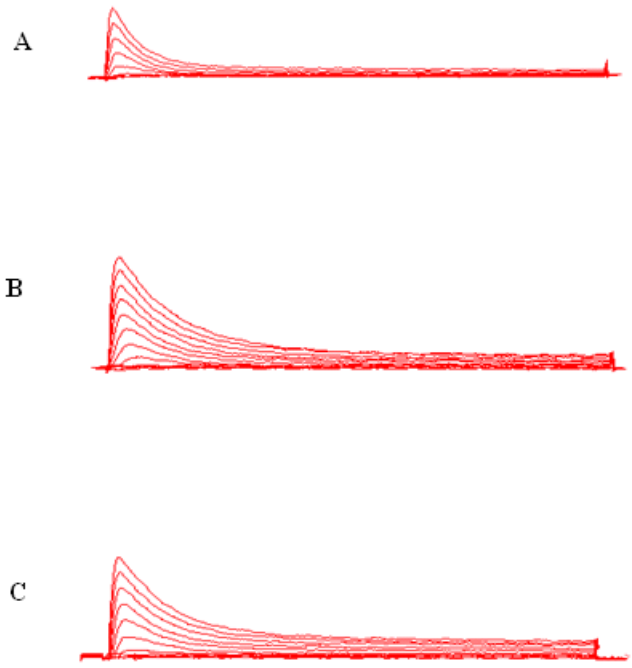

Figure 2. Representation of the ventricular current traces of Ito in the three rabbit groups. (A) MI group; (B) SHAM group; and (C) VAL group.

to membrane potential, are shown in the I-V curves (Figures 2 and 3).

DISCUSSION

Studies have shown that ventricular tissues undergo electrical remodeling and structure remodeling after MI, which can easily lead to malignant ventricular arrhythmia (St John Sutton et al., 2003). Laboratory experiments and clinical practice have proven that ACEIs reverse myocardial hypertrophy and its concomitant electrical remodeling (Huang et al., 2001; Li et al., 2004). AngII receptor antagonists block angII action at the receptor level through various ways. VAL is a highly selective and specific AngII receptor antagonist, and it can completely

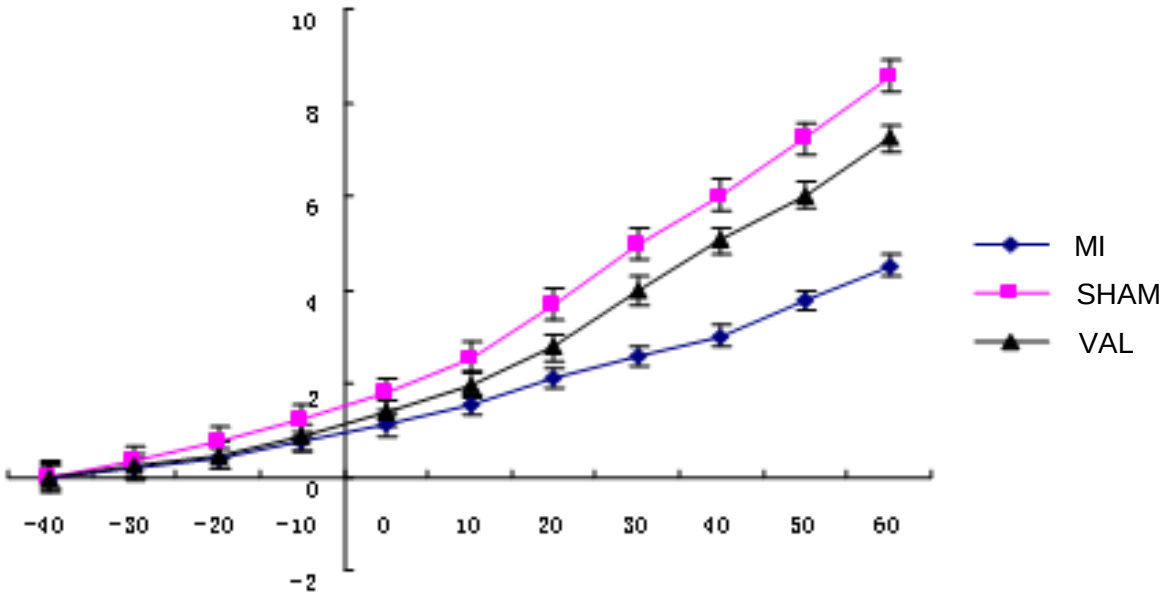

Figure 3. I-V curves of the Ito current in three rabbit groups. When the instructed voltage comes to +60 mV, the current density of Ito in SHAM, MI, and VAL groups were 8.56 ± 0.72, 4.51 ± 0.48, and 7.24 ± 0.68 pA/pF. The Ito current in the MI group was significantly lower than those in the SHAM group and the VAL group ($P < 0.05$).

block the AT1 receptor, which leads to activation-mediated AngII action. Some reports (Pfeffer et al., 2003) have shown that VAL blocks cardiac remodeling after MI, but few reports focused on the effects of VAL on the electrophysiology of the ventricle after MI. In the present study, we investigated the effects of VAL on the APD and Ito of ventricular myocytes after MI (Kimura et al., 2002).

Malignant ventricular arrhythmia is related to changes in ventricle regional repolarization. Our study demonstrated that the APD_{90} of the epicardium, mid-myocardium, and endocardium are lengthened after MI, especially the mid-myocardium. The difference was not statistically significant. This increases the heterogeneity of repolarization of the three ventricle muscular layers. Furthermore, the procedure stimulation could not induce ventricular arrhythmia before MI in the SHAM, the MI, or the VAL group. At 12 weeks after MI, VT/VF episodes were 11.7 ± 1.8 times in the MI group. This is the main mechanism that leads to malignant ventricular arrhythmia after MI, because of the increased heterogeneity of repolarization of the three-layered ventricle myocardium (Wirth et al., 2001; Chauhan et al., 2006).

Our research also shows that at 12 week after VAL treatment MI, the incidence of stimulation-induced malignant ventricular arrhythmia significantly decreased (only 3.2 ± 0.6 times); TDR clearly recovered when compared with the MI group, and was not significantly different from that in the SHAM group. The results demonstrate that VAL reduces the repolarization heterogeneity between ventricular muscle cells after MI, thereby decreasing the occurrence of malignant ventricular arrhythmia.

Ito is the ion flux during the repolarization of myocardial cell; it influences the earlier repolarization of the action potential. Ito also determines the electric potential of the early plateau of the action potential, and further influences the activity of other ion channels. Published studies have shown that Ito channel expression after MI and the density of Ito decreases (Oz et al., 2005). Decreased or absent Ito current density leads to prolongation of the plateau of the action potential and repolarization abnormalities. The Ito current was different between the MI and the non-MI regions, as well as in the different parts of the cardiac ventricle. The electrophysiologic heterogeneity increases the chance of recurrence, and it causes the recurrence of re-entrant ventricular arrhythmias after MI (An et al., 2006; Udyavar et al., 2008).

Our study shows that the Ito current density in the non-infarct region after MI evidently decreases. Furthermore, long-term VAL treatment after MI significantly decreases the density of Ito currents in non-infarct regions and reduces the ventricular repolarization abnormality after MI. The incidence of VF/VT is associated with electrical remodeling after MI. VAL inhibits electrical remodeling and decreases the incidence of VF/VT after MI.

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