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Effects of imidapril on heterogeneity of action potential and calcium current of ventricular myocytes in infarcted rabbits

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ABSTRACT

AIM: To investigate the effects of chronic treatment with imidapril on the electrophysiologic heterogeneous change of the noninfarcted myocardium of rabbits after myocardial infarction and the mechanism of its antiarrhythmic efficacy. **METHODS:** Rabbits with left coronary artery ligation were prepared and allowed to recover for 8 weeks. Myocytes were isolated from subendocardial, midmyocardial, and subepicardial regions of the noninfarcted left ventricular wall. Action potentials and calcium current were recorded using whole-cell patch clamp technique. **RESULTS:** The action potential duration of repolarization 90 % (APD₉₀) was more prolonged in midmyocardium rather than in subepicardium and subendocardium with healed myocardial infarction. The transmural dispersion of repolarization (TDR) was increased in the three ventricular regions. The amplitude of I_{Ca-L} was enhanced but its density was decreased in noninfarcted ventricular myocytes due to increased cell membrane capacitance. The increased differences of calcium currents among subepicardium, midmyocardium, and subendocardium were also discovered. Normalization of heterogeneous changes in repolarization after treatment with imidapril was observed and decrease of TDR in noninfarcted area was measured. Early after depolarization (EAD) events of noninfarcted midmyocardium were markedly decreased by imidapril. **CONCLUSION:** Imidapril reduced the electrophysiologic heterogeneities in noninfarcted area in rabbits after myocardial infarction. This ability of imidapril may contribute to its antiarrhythmic efficacy.

INTRODUCTION

The noninfarcted myocardium after healed myocardial infarction (HMI) underwent significant hypertrophy, which was considered as an adaptive universal response of the heart to increased workload from whatever causes^[1,2]. Although the cellular mechanisms of arrhythmogenesis in this pathophysiological condi-

tion were poorly understood, a high incidence of sudden death might be related to an abnormal prolongation of action potential duration (APD) and the enhanced transmural dispersion of repolarization (TDR)^[3], which was important factor of malignant ventricular arrhythmia, especially the *torsades de pointes*^[4].

Angiotensin-converting enzyme (ACE) inhibitor could prevent further deterioration in left ventricular function and the regression of hypertrophy in both human and animal models^[5,6], therefore, it was considered an important means to treat myocardial infarction. The exact mechanism of the protective effect of ACE

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remained unclarified. In order to investigate the impact of ACE inhibitor on the electrophysiologic heterogeneity, we studied the action potential and calcium currents of ventricular myocytes in HMI hearts, using the whole-cell patch clamp technique, then evaluated whether imidapril, an ACE inhibitor, could reverse the abnormal changes of repolarized heterogeneity in HMI hearts.

MATERIALS AND METHODS

Model of myocardial infarction A rabbit model of myocardial infarction used in this study was previously described^[7]. Rabbits (2.0 to 2.5 kg) were anesthetized with pentobarbital (30 mg/kg, iv) and the left anterior descending coronary artery was occluded. The rabbits were then allowed to recover for 8 weeks, as the healed myocardial infarction group (HMI group). Another group 3 d after surgery, received oral administration of imidapril (1.5 mg·kg⁻¹·d⁻¹) for 8 weeks, as the imidapril-treated group (IMI group). Animals undergoing an identical surgical procedure without coronary ligation served as the sham-operated group (sham group).

Isolation of myocytes The ventricular myocytes were isolated as described previously^[8]. The heart was suspended from a Langendorff column, and perfused with Tyrode's solution (in mmol/L) NaCl 135, KCl 5.4, CaCl₂ 1.8, MgCl₂ 1, NaH₂PO₄ 0.33, HEPES 10, and glucose 10 (pH 7.4). The enzymatic isolation solution contained collagenase 0.33 g/L, protease E 0.025 g/L, and bovine serum albumin 1.25 g/L (Sigma, Chemical Co) for 25 min. The subendocardium (Endo), midmyocardium (M), and subepicardium (Epi) of the noninfarcted ventricular region were sliced with a surgical blade. Each section was then minced and digested for 20 to 25 min in a fresh digestion solution. Cells were dispersed and stored at 20 °C.

Electrophysiological study The action potentials and currents were recorded at 37 °C using EPC-9 (HEKA, Germany). Stimuli output, data acquisition and processing were performed by Pulse-pulsefit software. Series resistance and capacitance were compensated and leak currents were subtracted. A compatible computer was connected to the amplifier via D/A and A/D converter. Micropipettes were made by using a two-stage puller (pp-83, Narishige) from star-bore capillary tubes (GG-17) and micropipettes had resistance from 2 to 4 MΩ when filled with the pipette solution of the following compositions (in mmol/L) CsCl 120, CaCl₂ 1, MgCl₂ 5, Na₂ATP 5, egtazic acid 11, HEPES 10, and

glucose 11 (pH 7.3). Voltage signals were low-pass filtered at 1 kHz by a 4-pole Bessel filter before sampling.

Under the current clamp configuration, the action potential was elicited by intracellular injection of depolarizing current (900 pA, 15-ms duration of 0.5 Hz) in cells superfused with normal Tyrode's solution. The pipette solution contained (in mmol/L) NaCl 120, CaCl₂ 1, MgCl₂ 5, Na₂ATP 5, egtazic acid 11, HEPES 10, and glucose 11 (pH 7.3). To record L-type calcium current (I_{Ca-L}), the cells were depolarized from a holding potential of -40 mV, to 150 ms different test potentials at 10 mV increased from -30 mV to +50 mV. It suggested that the current was L-type calcium current, which was slowly activated inward current and could be inhibited by nifedipine 5 μmol/L. This current was also enhanced by Bay-K-8644 0.5 μmol/L.

Statistics Data were expressed as mean±SEM. A *t*-test was applied to compare the results of two different groups and ANOVA was used for multiple comparisons. *P*<0.05 was considered statistically significant. The steady-state activation and inactivation of I_{Ca-L} were fitted by Boltzmann equation: $I/I_{max}=1/[1+\exp(V_{0.5}-V_m)/k]$. $V_{0.5}$ is instead of Half-maximal and *k* is instead of voltage and slope factor.

RESULTS

Histological changes of hearts Heart weight and heart weight to body weight ratios were significantly greater in HMI group than those in the sham group. The mean cell membrane capacitance in the noninfarcted myocytes from infarcted hearts was greater than those from sham hearts. With imidapril treatment, the structural changes of infarcted hearts were significantly alleviated (Tab 1).

Ventricular action potentials Action potential duration of myocytes in healed myocardial infarction area was significantly prolonged. A reduction of APD₂₀ in the subendocardium (*n*=20), midmyocardium (*n*=20), and subepicardium (*n*=19) was discovered in myocytes in healed myocardial infarction area. APD₉₀ in all layer cells was increased. Furthermore, prolongation of APD₉₀ of midmyocardium was more prominent than those of subendocardium and subepicardium in healed myocardial infarcted cells. TDR in healed myocardial infarcted myocytes was increased (*P*<0.01). Treatment with imidapril reversed these changes in action potential (Tab 2). However, there was no difference in resting membrane potential, action potential amplitude, or dv/dt_{max} in the three groups (Fig 1).

Tab 1. Characteristics of hearts and cell membrane capacitance of isolated myocytes in Sham, HMI, and IMI groups. Mean±SEM. ^b*P*<0.05, ^c*P*<0.01 vs sham group. ^e*P*<0.05, ^f*P*<0.01 vs HMI group.

	<i>n</i>	Sham	HMI	IMI
Heart weight/g	15	9.4±1.2	13.6±2.3 ^c	10.3±1.1 ^c
Heart weight/body weight/g·kg ⁻¹	15	2.91±0.13	3.8±0.3 ^b	3.13±0.17 ^c
Cell membrane capacitance/pF	45	154±12	223±13 ^b	168±10 ^f

Tab 2. APD values (ms) in subepicardium (Epi), midmyocardium (M), and subendocardium (Endo) after myocardial infarction. *n*=20. Mean±SEM. ^b*P*<0.05, ^c*P*<0.01 vs sham group. ^e*P*<0.05, ^f*P*<0.01 vs HMI group.

	Sham	HMI	IMI
Epi, APD ₂₀	141±48	105±15 ^b	128±15 ^e
APD ₅₀	195±45	202±52	209±53
APD ₉₀	223±50	313±41 ^b	253±41 ^f
M, APD ₂₀	150±46	111±17 ^b	139±34 ^e
APD ₅₀	275±48	283±56	299±38
APD ₉₀	304±32	454±56 ^c	352±22 ^f
Endo, APD ₂₀	133±25	118±16 ^b	126±17 ^e
APD ₅₀	168±13	182±18	180±16
APD ₉₀	243±21	286±14 ^b	248±18 ^f
TDR	82±17	168±20 ^c	104±14 ^f

Early afterdepolarization (EAD) EAD was observed in 16 out of 21 midmyocardiums, 8 out of 19 subepicardiums, and 7 out of 19 subendocardiums in the HMI heart. Graded increment of the slope and amplitude of the EAD in midmyocardium in HMI cells, which eventually reached a threshold and triggered spontaneous action potential. EAD was not found, in cells from the sham heart. EAD were detected in 5 out of 23 midmyocardiums, 2 out of 21 subepicardiums, and 2 out of 19 subendocardiums in imidapril-treated heart but failed to induce triggered activity (Fig 2).

L-type voltage-dependent calcium current (*I*_{Ca-L}) Typical ventricular *I*_{Ca-L} tracings from subepicardial, midmyocardial, and subendocardial regions in the three groups (Fig 3A). With the increment of cellular membrane capacitance in healed myocardial infarcted cells, *I*_{Ca-L} densities were decreased by 38.7 %, 35.2 %, and 41.0 % respectively in subepicardium, midmyocardium, and subendocardium (*P*<0.01). The average current densities of subepicardium, midmyocardium, and

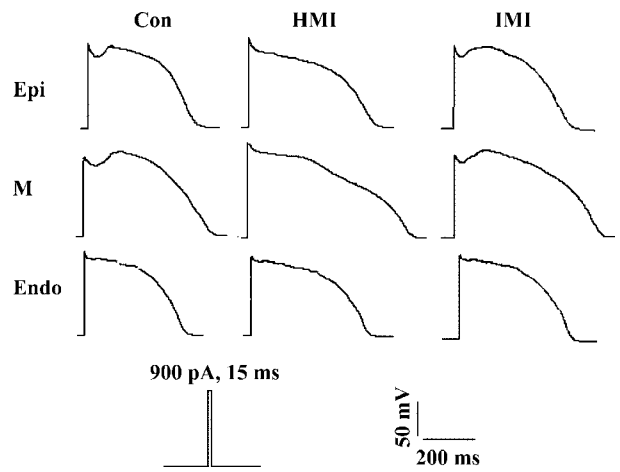


Fig 1. Comparison of action potentials of three layer myocytes in three group of rabbit hearts. TDR was significantly increased in HMI myocytes than those of sham myocytes. TDR in HMI myocytes was recovered by IMI treatment.

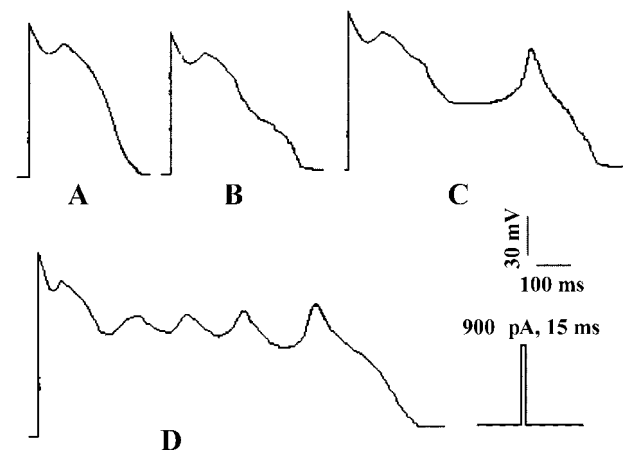


Fig 2. EAD of midmyocardium in HMI heart. Graded increase in the slope and amplitude of the EAD, which eventually reached a threshold level and triggered spontaneous APs (A-D).

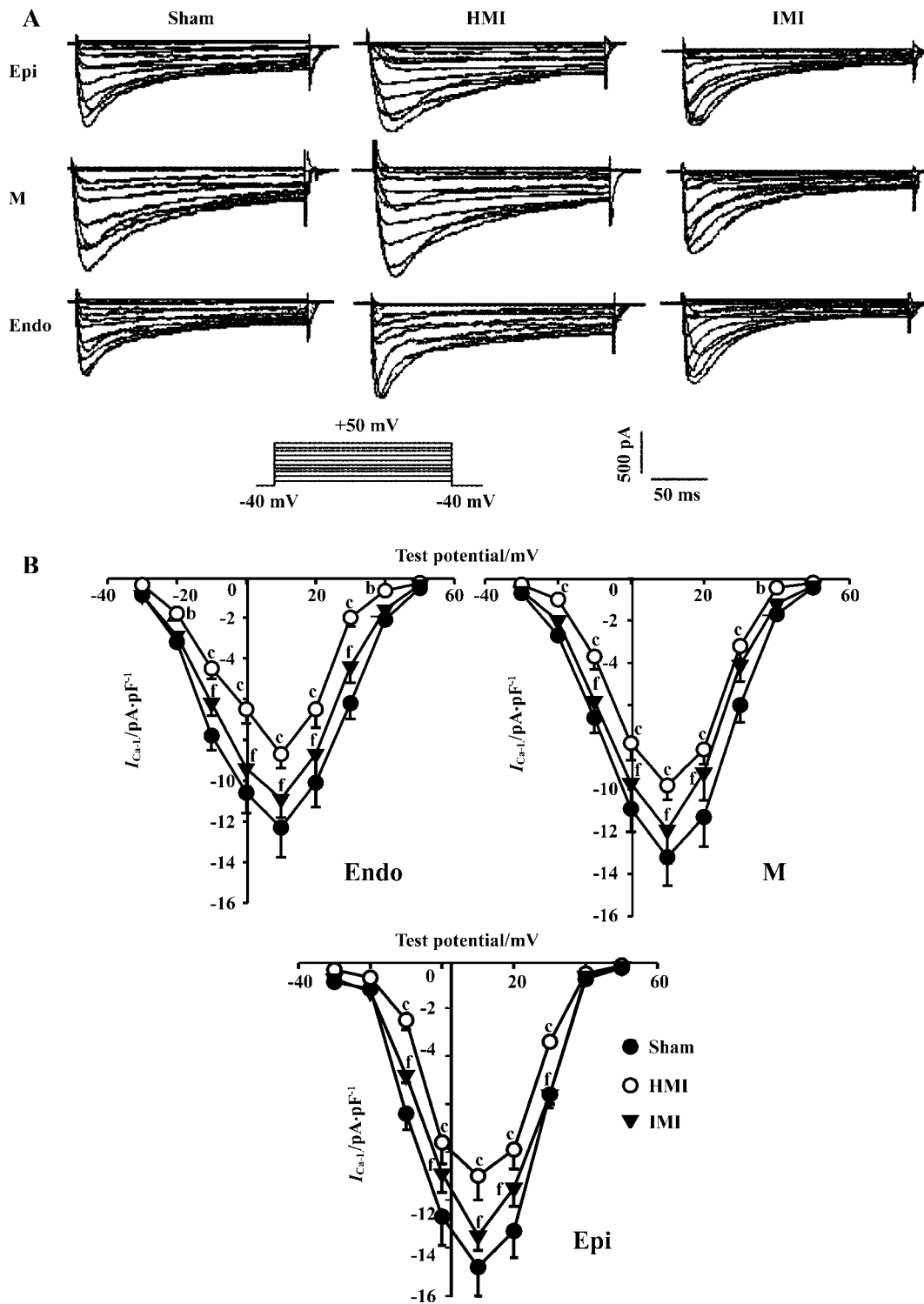


Fig 3. A) Representative ventricular current traces of I_{Ca-L} in three rabbit groups. B) $I-V$ curves showed that average densities of I_{Ca-L} in HMI cells were significantly different from those of sham and imidapril-treated cells. $n=18-22$. Mean \pm SEM. ^b $P < 0.05$, ^c $P < 0.01$ vs sham-operated groups. ^f $P < 0.01$ vs HMI.

subendocardium in healed myocardial infarcted cells were (9.2 ± 0.3) pA/pF, $n=18$; (9.8 ± 0.2) pA/pF, $n=20$; and (8.7 ± 0.3) pA/pF, $n=20$. After treatment with imidapril, the I_{Ca-L} densities were increased to $(11.5 \pm$

$0.6)$ pA/pF, $n=20$ (subepicardium), (11.2 ± 1.1) pA/pF, $n=22$ (midmyocardium), and (10.9 ± 0.4) pA/pF, $n=19$ (subendocardium). The mean current-voltage ($I-V$) relationship demonstrated that I_{Ca-L} densities on infarcted

myocytes were lower than those from sham-operated or imidapril-treated myocytes (Fig 3B). Half-maximal voltage ($V_{0.5}$) and slope factor (k) values obtained in both activation and inactivation curves were similar among the three regions. Time constants of recovery (τ_{rev}) in infarcted cells were slightly delayed, but the difference were not significant in the three groups.

Imidapril did not acutely affect either the shape of action potential or $I_{\text{Ca-L}}$ in all ventricular myocytes (data not shown).

DISCUSSION

This study showed different changes of APD from the noninfarcted ventricular area in three groups in response to cardiac infarction. A reduction of APD₂₀ in the subendocardium, midmyocardium, and subepicardium was discovered as a result of the decrease in $I_{\text{Ca-L}}$ density after healed myocardial infarction. APD₉₀, which should be decreased theoretically because of decrease in $I_{\text{Ca-L}}$ of HMI cells, was unexpectedly increased in all layers, furthermore, obvious augment of APD₉₀ was detected in midmyocardium instead of subendocardium and subepicardium. These results suggested that the intensity of the calcium current contributed greatly, but not exclusively, to this heterogeneity. Other currents such as transient outward potassium current, late sodium current, delayed rectifier potassium current, and special slowly activated component of delayed rectifier potassium current, were also involved in the changes of action potential^[9-11].

There were intrinsic electrical differences between myocytes from different regions of the heart, which were the result of different contributions of ionic currents to the transmembrane action potential. The electrical heterogeneity could be affected by different pathological conditions, such as myocardial ischemia and cardiac hypertrophy, which might be responsible for increased pro-arrhythmic events^[12]. In our study, $I_{\text{Ca-L}}$ density was decreased. It was not because of the altered steady state inactivation or its recovery from inactivation. The mechanism might be the decrease in the number of functional $I_{\text{Ca-L}}$ channels because of increase in cell membrane capacitance after healed myocardial infarction^[13].

Ang II, one of the most potent vasoconstrictors, was implicated in the development of various pathophysiological diseases. Ang II induced marked structural change of heart which resulted in myocardial hypertrophy and remodeling. Over the past decade, clinical

and laboratory studies provided evidences that interruption of the renin-angiotensin system by angiotensin-converting enzyme inhibitors might improve cardiac function, regress left ventricular remodeling, and prolong survival in patients with healed myocardial infarction. ACE inhibitors not only blocked the conversion of angiotensin I to angiotensin II, but also prevented the degradation of bradykinin and stimulated the production of prostaglandins that provided the vasodilation and hemodynamic benefits^[14]. This article reported that cells from infarcted rabbits treated with imidapril had nearly normal cell membrane capacitance, action potential duration, and calcium current density. Treatment with imidapril could decrease experimental animals' arrhythmia events and mortality after healed myocardial infarction, which might be caused either by restraining the constitution remodeling or regressing the electric remodeling^[15].

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