Effect of imidapril on heterogeneity of slow component of delayed rectifying $K^+$ current in rabbit left ventricular hypertrophic myocytes

LI Yang¹, LU Zai-Ying, XIAO Jian-Men, MA Jie, NIU Hui-Yan, LIU Nian, RUAN Yan-Fei

Department of Cardiology, Tongji Hospital Affiliated to Tongji Medical College, Huazhong Science and Technology University, Wuhan 430030, China

KEY WORDS  imidapril; left ventricular hypertrophy; potassium channels

ABSTRACT

AIM: To investigate the transmural heterogeneous change of slow component of delayed rectifying potassium current in rabbit left ventricular hypertrophic myocytes and the effect of long-term treatment with imidapril (Imi).

METHODS: Rabbits were divided into hypertrophy group (left ventricular hypertrophy induced by partial ligation of abdominal aorta), Imi-treated group (surgical treatment as hypertrophy group was treated with Imi), and Sham-operated group as control. Whole-cell patch-clamp technique was used to record potassium currents.

RESULTS: (1) Membrane capacitance was larger in hypertrophic cells than in sham-operated and Imi-treated cells. Action potential durations (APD) of epicardium (Epi), midmyocardium (M), and endocardium (Endo) were remarkably longer in hypertrophic cells than those in Imi-treated and sham-operated cells. The prolongation of APD of M was the most pronounced in three layer myocytes of hypertrophic group. (2) The densities of $I_{Ks,tail}$ of hypertrophic cells were reduced by Epi 25.3 %±2.9 %, M 38.0 %±3.7 % and Endo 20.3 %±4.7 % compared with those of sham-operated cells. The decrease of $I_{Ks,tail}$ density was more pronounced in M than in Epi or Endo ($n = 13$, $P<0.01$ vs Epi or Endo). (3) The density of $I_{Ks,tail}$ in Imi-treated cells was not different from that in sham-operated cells significantly ($n = 10$). CONCLUSION: Imi reduced prolongation of APD and inhibited the heterogeneous change of $I_{Ks,tail}$ in rabbit left ventricular hypertrophic myocytes.

INTRODUCTION

Left ventricular hypertrophy (LVH) is associated with an increased risk of malignant ventricular arrhythmia and sudden cardiac death. The increased vulnerability to ventricular arrhythmia appears to be the result of action potential prolongation and altered repolarization¹.². Heterogeneities in the distribution of repolarizing currents among the ventricular wall have been identified in normal heart. In several species including dog, ferret, and rabbit, as well as in humans, the distribution of slow component of delayed rectifying potassium current ($I_{Ks}$) has been found to be significantly different in sub-endocardial (Endo), midmyocardial (M) and sub-epicardial (Epi) regions. Recently, it is demonstrated that cardiac hypertrophy causes a more pronounced increase of APD in myocytes from M than in those from Endo or Epi layer of the left ventricular free wall. It suggests that these regional change in APD may be responsible for the changes in ECG and the ventricular...
Arrhythmia events in hypertrophy[3].

Angiotensin II is the key signal substance for myocyte hypertrophy and it has been demonstrated that angiotensin-converting enzyme inhibitor (ACE inhibitor) is able to reduce the development of hypertrophy and the incidence of arrhythmia[5]. ACE inhibitors are known to be effective antihypertensive drugs for reducing left ventricular hypertrophy in hypertension[5]. Imidapril (Imi), a kind of ACE inhibitor, can cause marked regression of ventricular hypertrophy[6]. The effects of Imi on change of transmural heterogeneity of slow component of delayed rectifying potassium current remain to be investigated. The present study is designed to determine whether Imi can reverse abnormal heterogeneity change of slow component of delayed rectifying potassium current in LVH.

MATERIALS AND METHODS

Agents and animals Collagenase type I, protease E, bovine serum albumin, egtazic acid, K₂ATP, CdCl₂, MgCl₂, NaCl, NaOH, N-[2-hydroxyethyl]piperazine-N'-[2-ethane-sulfonic acid] (HEPES), 4-aminopyridine (4-AP), Na-pyruvate and K-aspartate were purchased from Sigma Co, dofetilide from Pfizer Co, tetrodotoxin (TTX) from Hebei Aquatic Product Research Institute, imidapril (Imi) was provided as a gift from Tanabe Seiyaku Co Inc, other reagents are of analytical grade. Male or female rabbits weighing 2.0-2.5 kg were provided by the Experimental Animal Center of Tongji Medical College of Huazhong University of Science and Technology (Certificate No 19-020).

Surgical procedure Body weight and systolic blood pressures were measured from all rabbits before initiation of the experiment. Animals were divided into three groups: hypertrophy group, cardiac hypertrophy group, and control group, these animals only underwent the abdominal laparotomy without further procedure. Animals in the hypertrophy group, on the next day of surgery as hypertrophy group, the rabbits were treated with oral administration of Imi (1.5 mg·kg⁻¹·d⁻¹) for 8 weeks; Sham-operated as control group, these animals only underwent the abdominal laparotomy without further procedure.

Cell preparation Single ventricular myocytes of rabbits were isolated by enzymatic dissociation method similar to that previously described[7]. The Endo and Epi tissues were separated from other region (M) visually with a razor. Single cells in each region were obtained by gentle shaking and dispersed in a chamber filled with normal Tyrode’s solution.

Electrophysiologic recording Single cells were transferred to a chamber mounted on the stage of an inverted microscope. The currents were recorded at 37 °C with the whole cell voltage-clamp configuration using EPC-9 (HEKA, German). Stimuli output or data acquisition and processing were performed by pulse-pulsefit software, and a compatible computer was connected to the amplifier via D/A and A/D converter. Micropipettes were made of a two-stage puller (pp-83, Narishige) from star-bore capillary tubes (GG-17) and had resistance of 2-4 MΩ. Voltage signals were low-pass filtered at 1 kHz by a 4-pole Bassel filter before sampling.

To record the action potential, the cells were superfused with normal Tyrode’s solution (mmol/L: NaCl 135, KCl 5.4, CaCl₂ 1.8, MgCl₂ 1, NaH₂PO₄ 0.33, HEPES 10, glucose 10, pH was adjusted to 7.4 with NaOH). The pipette internal solution for the action potential contained (mmol/L): NaCl 120, CaCl₂ 1, MgCl₂ 5, Na₄ATP 5, egtazic acid 11, HEPES 10, glucose 11, and pH was adjusted to 7.3 with NaOH.

The extracellular solution for I_k currents measurements was NMG solution (mmol/L: N-methyl-D-glutamine 149, MgCl₂ 5, HEPES 5). In this Na⁺-free, K⁺-free, CdCl₂ 100μmol/L-contained and 4-AP 5 μmol/L-contained external solution, pH was adjusted to 7.4 with HCl. The I_caL, I_k1, I_m, the Na⁺-Ca²⁺ exchange current, and the Na⁺-K⁺ pump current were negligible in this solution. The pipette internal solution for measuring I_k current contained (mmol/L): K-aspartate 85, KCl 45, Na-pyruvate 5, K₇-ATP 3, MgCl₂ 4, egtazic acid 10, HEPES 10, d-glucose 11 and pH was adjusted to 7.3 with KOH.

Data analysis Data were expressed as mean±SD and n represents the number of cells. Statistical significance was determined by t-test and ANOVA.

RESULTS

Membrane capacitance in the three-LV wall myocytes The body weights, blood pressures, heart weight, and ventricular wall thickness were assessed in the three groups, respectively. Membrane capacitance was measured by applying a 0.4-V/s ramp protocol from a holding potential of −80 mV and calculated according to the equation: C_m=I/dv·dr⁻¹, where C_m is the membrane capacitance, I is the amplitude of the capacitance current, dv·dr⁻¹ is the slope of the voltage pulse. As shown in Tab 1, cell capacitance of hypertrophied rabbits was significantly increased compared with that of
In the study, to get rid of the effect of sham-operated rabbits (n=45 cells from 10 animals, P<0.01), while cell membrane capacitance in Imi-treated cells was similar to that of sham-operated cells. In the study, to get rid of the effect of C_{m} on the current amplitude, we compared the densities of currents in the three groups.

**Effect of Imi on the action potential of myocytes from the three layers of LV wall** Under the current clamp circumstance, the action potential was elicited by applying 900-pA, 15-ms duration stimuli at frequency of 0.5 Hz. As shown in Fig 1, there was longer APD_{90} in the hypertrophy cells than those of sham-operated (n=13 cells from 7 animals, P<0.01) and Imi-treated cells (n=13 cells from 6 animals, P<0.01). This prolongation of APD_{90} was more pronounced in M than Epi and Endo myocytes (n=13 cells from 7 animals, P<0.01 vs Epi or Endo) in the hypertrophy cells and transmural repolarization heterogeneity was increased significantly. The heterogeneous changes of APD_{90} in the hypertrophy group were reversed in Imi-treated group, which was similar to that of sham-operated group (Fig 1, Tab 2).

**Tab 1. Characteristics of rabbit heart in sham-operated, LVH, and Imi-treated groups. Mean±SD. *P<0.05, †P<0.01 vs sham-operated group. ‡P<0.05, §P<0.01 vs hypertrophy group.**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Sham-operated</th>
<th>Hypertrophy</th>
<th>Imi-treated</th>
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<tbody>
<tr>
<td>Mean arterial pressure/mm Hg</td>
<td>10</td>
<td>84±5</td>
<td>135±12*</td>
<td>102±8*</td>
</tr>
<tr>
<td>Heart weight/g</td>
<td>10</td>
<td>9.4±1.2</td>
<td>13.6±2.3†</td>
<td>10.3±1.1†</td>
</tr>
<tr>
<td>Heart weight/body weight/g·kg⁻¹</td>
<td>10</td>
<td>2.91±0.13</td>
<td>3.8±0.3‡</td>
<td>3.13±0.17‡</td>
</tr>
<tr>
<td>Ventricular wall thickness/mm</td>
<td>10</td>
<td>3.24±0.19</td>
<td>4.5±0.4§</td>
<td>3.59±0.18§</td>
</tr>
<tr>
<td>Cell membrane capacitance/pF</td>
<td>45</td>
<td>154±12</td>
<td>203±21⁄</td>
<td>168±15‡</td>
</tr>
</tbody>
</table>

**Fig 1. Effect of imidapril on the action potentials in the three-LV wall myocytes in rabbit hypertrophy hearts. (frequency of 0.5 Hz). n=13 cells from 7 animals (sham-operated and LVH group), n=13 cells from 6 animals (Imi-treated group).**

**Tab 2. APD_{90} of three-layer myocytes in sham-operated, LVH, and Imi-treated groups. n=13. Mean±SD. *P<0.05, †P<0.01 vs sham-operated or Imi-treated groups. ‡P<0.05 vs Epi or Endo.**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>LVH</th>
<th>Imi</th>
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<tbody>
<tr>
<td>Epi</td>
<td>351±19</td>
<td>435±23†</td>
<td>373±25</td>
</tr>
<tr>
<td>M</td>
<td>478±42‡</td>
<td>619±41§</td>
<td>492±43‡</td>
</tr>
<tr>
<td>Endo</td>
<td>327±34</td>
<td>421±32‡</td>
<td>355±37</td>
</tr>
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</table>

**Effect of Imi on I_{Ks} current of myocytes from the three layers of LV wall** Cells were exposed to dofetilide 1 μmol/L to block I_{Kr}. I_{Ks} and I_{Ks,tail} were recorded by applying various voltage pulses ranging from -20 mV to +50 mV for 7 s from holding potential of -50 mV, followed by repolarizing to -30 mV. At test potential of +50 mV, the densities of I_{Ks,tail} in the sham-operated cells were (0.8±0.1) pA/pF (Epi), (0.4±0.1) pA/pF (M), and (0.8±0.1) pA/pF (Endo). The densities of I_{Ks,tail} in hypertrophic cells were (0.4±0.1) pA/pF (Epi), (0.2±0.03) pA/pF (M), and (0.4±0.03) pA/pF (Endo) [reduced by 25.3±2.9% (Epi), 38.0±3.7% (M) and 20.3±4.7% (Endo) vs sham-operated cells]. The extent of the decrease of I_{Ks,tail} density in hypertrophic cells was more pronounced in M than that in Epi and Endo (n=13 cells from 5 animals, P<0.01 vs Epi or Endo). The densities of I_{Ks,tail} in Imi-treated cells were enhanced to (0.8±0.1) pA/pF (Epi), (0.3±0.03) pA/pF (M), and (0.3±0.02) pA/pF (Endo).
Effect of Imi on $I_{K_s}$ current-voltage relationship of myocytes from the three layers of LV wall

$I_{K_s}$ and $I_{K_s,tail}$ were recorded by applying various voltage pulses ranging from -20 mV to +50 mV for 7 s from holding potential of -50 mV, followed by repolarizing to -30 mV. Current-voltage relationship of $I_{K_s,tail}$ in the three-LV wall myocytes in the three groups were shown in Fig 3.

Effect of Imi on $I_{Kr}$ current of myocytes from the three layers of LV wall

$I_{Kr}$ was recorded during 225-ms depolarizing pulse to 0 mV from a holding potential of -50 mV, and the tail current ($I_{Kr,tail}$) was determined upon repolarization to -40 mV, which could be blocked almost completely by dofetilide 1 µmol/L. The densities of $I_{Kr,tail}$ in the hypertrophic myocytes were similar to those of sham-operated and Imi-treated cells ($n=10$ cells from 4 animals, $P>0.05$). The densities of $I_{Kr,tail}$ were also not strikingly different in Epi, M and Endo cells in each group ($n=10$ cells from 4 animals, $P>0.05$) (Fig 4).

Direct effects of Imi on APD and $I_{K}$ of myocytes from the three layers of LV wall

To determine whether any of the change in membrane current densities observed in the experiment was due to a direct electrophysiological effect of Imi, we examined the AP and current-voltage relationship of $I_{K_s}$ and $I_{K}$ before and after the cells were exposed to Imi 10 µmol/L in myocytes from sham and LVH rabbits. We found that application of Imi had no significant effect on APD and $I_{K}$ of control myocytes. Similar results were also obtained from the hypertrophied myocytes.

DISCUSSION

Hypertension is one of the major underlying diseases leading to heart failure. For more than 20 years, regression of ventricular hypertrophy has been a major goal of clinical treatment and of hypertension research. The renin-angiotensin system plays an important role in the regulation of cardiac myocyte growth. ACE inhibitors have been shown to reduce left ventricular (LV) weight significantly[8].

Our data showed that $I_{K_s}$ amplitude was small in hypertrophied ventricular myocytes and its current density was even smaller than that of sham cells, indicating that channel numbers decreased at enlarged myocytes. Cardiac hypertrophy altered the regional distribution of action potential duration by affecting the magnitude and kinetics of underlying ionic currents[9]. The reduction of $I_K$ led to a repolarizing delay in myocytes[10].

Another interesting finding in our data was that in hypertrophic myocytes the prolongation of APD and the decrease of $I_{K}$ density were much more evident in midmyocardial than in epicardial and endocardial myocytes. It was thought to be responsible for the transmural dispersion of refractoriness and increased vulnerability to ventricular arrhythmia. Therefore, the reduction of $I_{K_s}$ in hypertrophied myocytes should be recognized as a significantly electrical remodeling[11].

This study also demonstrated normalization of cell membrane capacitance and $I_{K_s}$ current abnormalities of ventricular myocytes after regression of LVH with...
chronic Imi treatment. Furthermore, normalization of $I_{Ks}$ current abnormality was associated with normalization of APD, which probably explains the less transmural heterogeneity of repolarization after Imi treatment than that of LVH cells.

The results showed that Imi had no direct effect on membrane currents of either control or LVH myocytes. However, myocytes of LVH rabbits treated with Imi for a long term had normal cell membrane capacitance, membrane current density, and action potential duration. Those were probably responsible for the reduced vulnerability to ventricular arrhythmia observed in LVH model after treatment with Imi.

REFERENCES
