The effect of vimentin on cardiac contractility, calcium dynamics and arrhythmia in myocardial ischemia reperfusion injury of rats

Hee-Sun Mun

Department of Medicine

The Graduate School, Yonsei University

The effect of vimentin on cardiac contractility, calcium dynamics and arrhythmia in myocardial ischemia reperfusion injury of rats

Directed by Professor Boyoung Joung

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Hee-Sun Mun

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연세대학교
YONSEI UNIVERSITY

This certifies that the Doctoral Dissertation of Mun Hee-Sun is approved.

Thesis Supervisor : Boyoung Joung

Thesis Committee Member#1 : Bae Hwan Lee

Thesis Committee Member#2 : Hui-Nam Pak

Thesis Committee Member#3: Chul Hoon Kim

Thesis Committee Member#4: Ji Hyung Chung

The Graduate School
Yonsei University

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<TABLE OF CONTENTS>

ABSTRACT ····· 1

I. INTRODUCTION ····· 3

II. MATERIALS AND METHODS ····· 5
1. Ischemia-Reperfusion injury in rats ····· 5
2. Optical mapping ····· 6
3. Programmed stimulation ····· 7
4. Primary culture of neonatal cardiomyocytes ····· 8
5. Dynamic Ca^{2+} imaging ····· 8
6. Statistical analysis ····· 9

III. RESULTS ····· 10
1. Ischemia-Reperfusion injury and Vimentin treatment ····· 10
2. Effects of vimentin on the Electrophysiologic Characteristics ····· 12
3. The effect of vimentin on calcium transient and contractility after the Ischemia-Reperfusion injury ····· 15

IV. DISCUSSION ····· 17

V. CONCLUSION ····· 19

REFERENCES ····· 20
ABSTRACT(IN KOREAN) ····· 23

LIST OF FIGURES

Figure 1. Diagram of the study design ····· 6

Figure 2. The assessment of the infarction among 3 groups by histology ····· 10

Figure 3. The comparison of arrhythmia among 3 groups ···· 11

Figure 4. The action potential duration among 3 groups ····· 12

Figure 5. The conduction velocity and heterogeneity among 3 groups ····· 14

Figure 6. The calcium transient among 3 groups ····· 15

ABSTRACT

The effect of vimentin on cardiac contractility, calcium dynamics and arrhythmia in myocardial ischemia reperfusion injury of rats

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Department of Medicine
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Vimentin, an intermediate filament, is the cytoskeletal component responsible for maintaining cell integrity. The extensive T-tubular system of ventricular myocytes ensures a rapid and homogeneous increase in $[Ca^{2+}]i$ throughout the cell. This study investigated whether externally administered vimentin could improve cardiac contractility and Ca^{2+} release by stabilizing cardiomyocyte integrity in ischemia-reperfusion (I/R) injury of rats model.

I/R injury in rats were produced by the ligation of the left anterior descending artery for 1 hour followed by reperfusion for 3 hours in Sprague–Dawley rats (I/R group, n=10). One hour after the I/R injury, 125 μL of vimentin solution (0.4 μg/μL) were systemically injected via a leg vein (I/R+Vimentin group, n=10). During hearts were perfused, maps were optically analyzed action potential duration (APD), conduction velocity (CV), restitution kinetics and the vulnerability of ventricular arrhythmias. The contractility was evaluated using adult cardiomyocytes of the I/R group and the I/R+Vimentin group normalized by control. Neonatal rat cardiac myocytes were prepared from hearts of 1-3 day old Sprague-Dawley rats, and dynamic Ca^{2+} images were determined by measuring fluorescent intensity in hypoxia treated cells with and without vimentin using confocal microscope.

Compared with control, myocardial infarction was confirmed with respect to the infarct size of left ventricle both in I/R and I/R+Vimentin group (21.3 ± 1.4 % and 19. 8 ± 1.1 %, $p<0.01$, respectively). The cardiomyocyte contraction was decreased in hypoxia condition by 0.5 ± 0.2 ($p<0.05$). However, after cotreatment with vimentin, the contractility was recovered nearly upto control level (1.0 ± 0.9 vs. control, p=N.S.). Compared with control (1.0 ± 0.0 F/F0), the amplitudes of the Ca^{2+} waves were markedly increased in both I/R group (4.8 ± 1.0 F/F0, $p<0.01$) and I/R+Vimentin group (4.9 ± 1.1 F/F0, $p<0.01$). The frequencies of the Ca^{2+} waves were not different among 3 groups (1.0 ± 0.0 Hz in control, 0.9 ± 0.5 Hz in I/R group and 1.1 ± 0.3 Hz in I/R+Vimentin group, p=1.0). While spontaneous ventricular arrhythmias were not observed in control rats, they occurred frequently in I/R group (6 out of 10, 60 %) and in I/R+Vimentin group (5 out of 7, 71 %). APD was shortened, and the steepness of the CV restitution slope was increased in both I/R and I/R+Vimentin group.

Externally administered vimentin improved cardiac contractility after hypoxic insult. However, Vimentin made no change of the Ca^{2+} overloading in neonatal cardiomyocyte and more frequent ventricular arrhythmias after I/R injury. This result suggested that vimentin might be used as a therapeutic tool to increase cardiac contractility. However, arrhythmogenic effect should be further evaluated.

Key words : vimentin, ischemia reperfusion, arrhythmia, calcium

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I. INTRODUCTION

Intermediate filaments (IFs) are major components of the cytoskeleton and nuclear envelope. They are characterized by 10 nm diameter and marked insolubility.[1] Vimentin is a IF protein and the major cytoskeletal component of mesenchymal cells. Vimentin is attached to the nucleus, endoplasmic reticulum, and mitochondria, either laterally or terminally.[2] Vimentin plays a significant role in supporting and anchoring the position of the organelles in the cytosol. The dynamic nature of vimentin is important when offering flexibility to the cell. Scientists found that vimentin provided cells with a resilience absent from the microtubule or actin filament networks, when under mechanical stress *in vivo*. Therefore, in general, it is accepted that vimentin is the cytoskeletal component responsible for maintaining cell integrity.[3]

In heart muscle cells, the process of excitation–contraction (EC) coupling is mediated by Ca^{2+} influx through sarcolemmal L-type Ca^{2+} channels activating Ca^{2+} release channels (ryanodine receptors, RyRs) in the sarcoplasmic reticulum

(SR).[4, 5] Sarcolemmal L-type Ca^{2+} channels, dihydropyridine receptors and RyRs are organized in junctional complexes (couplons), which are predominantly found in the T tubules.[6, 7] Ca^{2+} sparks represent elementary events during normal EC coupling in cardiac muscle. In ventricular myocytes, Ca^{2+} sparks occur at Z lines near T tubules, probably at the junctional complex.[8-10] The extensive T-tubular system of ventricular myocytes ensures a rapid and homogeneous increase in $[Ca^{2+}]$i throughout the cell. It was reported that areas of delayed release are related to regional absence of T tubules but not RyRs in ventricular myocytes. This lower number of functional couplons contributes to a slower overall rate of rise of $[Ca^{2+}]_i$.[11] In arrhythmia such as persistent AF, reduced SR Ca^{2+} release despite preserved SR Ca^{2+} content is a major factor in contractile dysfunction. Fewer Ca^{2+} channel–RyR couplings and reduced efficiency of the coupling at subsarcolemmal sites, possibly related to increased Na^+ / Ca^{2+} exchanger, underlie the reduction in Ca^{2+} release.

This study attempted to investigate whether vimentin, which is responsible for maintaining cell shape, integrity of the cytoplasm, and stabilizing cytoskeletal interactions, could influence myocardial calcium release and contraction using rat ischemia-reperfusion (I/R) injury hearts. In addition, the influence of vimentin on arrhythmia was examined.

II. MATERIALS AND METHODS

This investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and was approved by the Institutional Animal Care and Use Committee of Yonsei University Health System.

1. Ischemia-Reperfusion injury in rats

Surgical myocardial infarctions were induced in 8-week-old male Sprague-Dawley rats (240 ± 10 g). The rats were anesthetized by an intramuscular injection of ketamine hydrochloride (80 mg/kg) and xylazine hydrochloride (4 mg/kg). The anesthetized rats were ventilated with positive pressure (180 mL/min) using a Harvard ventilator (Harvard Apparatus, Millis, MA, USA). A 2 cm incision was placed at the left lateral costal rib to expose the heart. The left anterior descending (LAD) artery was ligated for 1 hour with a 6-0 silk suture (Ethicon, Somerville, NJ, USA), followed by reperfusion for 3 hours. The ischemia was confirmed by visual inspection of blanching in the myocardium distal to the site of the occlusion.

After a one hour LAD ligation, 125 μL of a vimentin solution (0.4 μg/μL) in phosphate buffered saline was systemically injected via the leg veins of the rats (Figure 1). The rats (240 ± 10 g) were randomly divided into the following 3 groups: 1) control (sham operation), 2) I/R (I/R injury and saline treatment), and 3) I/R+Vim (I/R injury and vimentin injection). In the sham-operated rats, the same procedure was performed without an LAD ligation and reperfusion.

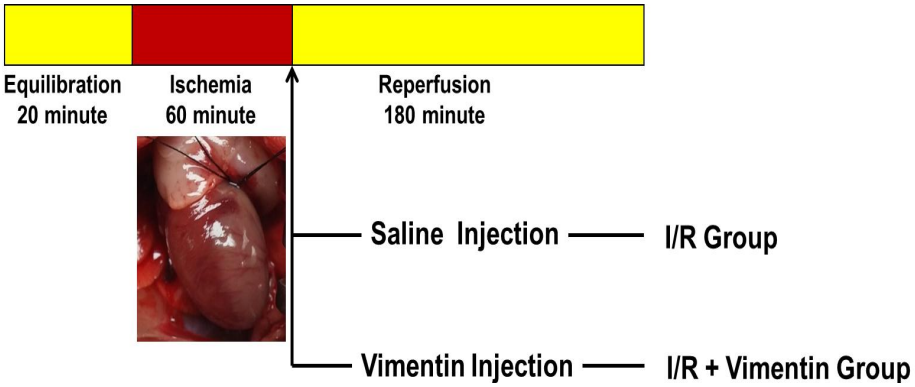

Figure 1. Diagram of the study design. Ischemia-reperfusion (I/R) injury were produced by the ligation of the left anterior descending artery for 1 hour followed by reperfusion for 3 hours in Sprague–Dawley rats. One hour after the I/R injury, saline was injected in I/R group and 125 μL (0.4 μg/μL) of vimentin solution in I/R+Vimentin group systemically via a leg vein.

2. Optical mapping

The rats (250 – 300 g) were anesthetized with ketamine (80 mg/kg) and xylazine (4 mg/kg), injected with heparin (200 U/kg IV), and then the heart was excised and perfused with a Langendorff apparatus with physiological Tyrode's solution containing (in mmol/L): 122 NaCl, 25 $NaHCO_3$, 4.81 KCl, 2 $CaCl_2$, 2.75 $MgSO_4$, and 5 Glucose (pH 7.4) gassed with 95 percent O_2 and 5 percent CO_2 at 37.0 ± 0.2 °C. The hearts were placed in a chamber and perfused with blebbistatin (3 - 5 μM) for 10 min to reduce the motion artifacts.[12] The hearts were stained with rhod-2 AM (Invitrogen, Grand Island, NY, USA) for calcium and RH237 (Invitrogen, Grand Island, NY, USA) for voltage imaging. Fluorescence was collected using dual cameras (BrainVision, Tokyo, Japan) at 1,024 frames/sec. The pixel resolution was 150 x 150 μm^2, and the data was

recorded and stored at intervals of 4-8 seconds.

Activation and repolarization time points at each site were determined from fluorescence (F) signals by calculating (dF/dt) the maximum and 90% recovery to baseline (APD_{90}). The mean APD_{90} was calculated for each heart by averaging APD_{90} from a minimum of 5 hearts in each group. The transmembrane potential (V_m) and intracellular calcium (Ca_i) transient were normalized to their respective peak-to-peak amplitude for a comparison of the timing and morphology. The local conduction velocity (CV) vectors were calculated for each pixel from the differences in the activation time-points of that pixel (determined from $(dF/dt)_{max}$) and its 7 × 7 nearest neighbors, as previously described.[13, 14] Local CVs were averaged and calculated as the means ± standard deviation (SD). To avoid any overestimations of the CVs, CVs > 1.25 m/s were deleted from the analysis.[14] The APD and CV restitution Kinetics (RK) curves were generated from the S_1-S_2 stimulation protocol.

3. Programmed Stimulation

To test the vulnerability of ventricular tachyarrhythmias, each heart was paced from the left ventricle using a programmed stimulation protocol consisting of a 20 S_1 pulse cycle length (CL) of 300 ms followed by a premature S_2 pulse with progressively shorter S_1-S_2 interval steps: 300 to 100 ms in 20 ms steps; 100 to 70 ms in 10 ms steps, and 60 to 35 in 5 ms steps, until loss of capture or the initiation of ventricular tachyarrhythmias. Optical mapping and induction studies of ventricular tachyarrhythmias were performed in 5 rats in each group.

4. Primary culture of neonatal cardiomyocytes

Neonatal rat cardiac myocytes were prepared from hearts of 1 - 3 day old Sprague-Dawley rats. Ventricles were trisected, pooled and enzymatically digested in collagenase. After a pre-plating purification step, cells were plated for 16-18 h in a solution of Dulbecco's modified Eagle's medium containing 10 % (v/v) horse serum, 5 % (v/v) heated-inactivated fetal calf serum, BrdU 100 mM and penicillin/streptomycin (100 units/ml). Cells were plated at a final density of 1-5x10^3/mm^2 on culture plates. Recombinant human TNF-α (Sigma Aldrich, Schnelldorf, Germany) was used in this study.

The hypoxic environment was generated using a hypoxia chamber. Briefly, neonatal cardiomyocytes were divided in to the following groups: (i) Control, cultures in normal growth media were placed in the chamber and flushed with a mixture of gases (95 % O_2, 5 % CO_2), (ii) Hypoxia/Reoxygenation (H/R), cultures in normal growth media were placed in the chamber and flushed with a mixture of gases (94 % N_2, 5 % CO_2, and 1 % O_2) for 1 hour, then were placed in the chamber (95 % O_2, 5 % CO_2) for 3 hours, and (iii) H/R+Vimentin, comprised of a treatment with vimentin solution (0.4μg/μL) for 24 h prior to the H/R in normal growth media.

5. Dynamic Ca^{2+} imaging

Images were obtained using a confocal microscope (LSM700, Carl Zeiss, Germany). Cardiac myocytes were washed three times with live cell imaging solution (140 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 20 mM HEPES-Na, 5.6 mM glucose, pH 7.4) and loaded with 5 μM fluo-4AM for 20

min at 37°C. Coverslips were mounted in 1 ml capacity chambers and placed in the microscope for fluorescence measurements after excitation with a 488-nm wavelength argon laser beam or filter system. Fluo-4 will be excited by 488 nm line of an Argon laser and emission signals over 505 nm will be collected. Line-scan images are acquired along the longitudinal axis of the cell. Each line comprises 512 pixels spaced at 0.14 μm intervals. After a sequential scanning, a two-dimensional image of 512 x 10000 lines or 512 x 20000 lines will be generated and stored for offline analysis. Image sequences were analyzed using the NIH open-access software ImageJ. Intracellular Ca^{2+} levels are expressed as the percentage of fluorescence intensity relative to basal fluorescence.

6. Statistical analysis

Continuous variables are reported as mean ± SD. Analysis of variance with post hoc test was used to compare the means of continuous variables that are approximately normally distributed among groups. Categorical variables are reported as count (percentage) and are compared using Fisher's exact test. The SPSS statistical package (SSPS Inc., Chicago, Illinois, USA) was used to perform all statistical evaluations. A p value of ≤ 0.05 was considered statistically significant.

III. RESULTS

1. Ischemia-Reperfusion injury and Vimentin treatment

To evaluate the effects of the vimentin against I/R injury, the extent of the infarct size was assessed by histology with triphenyl tetrazolium chloride (TTC) staining (Figure 2A). In immunohistochemical photograms, both in I/R and I/R+Vimentin group, derangement and hemorrhage were observed by hematoxylin-eosin(H-E) staining (Figure 2B) and focal necrosis by masson trichrome(M/T) staining (Figure 2C). Compared with control, myocardial infarction was confirmed with respect to the infarct size of left ventricle both in I/R group (21.3 ± 1.4% vs. control, $p<0.01$) and I/R+Vim group (19.8 ± 1.1% vs. control, $p<0.01$).

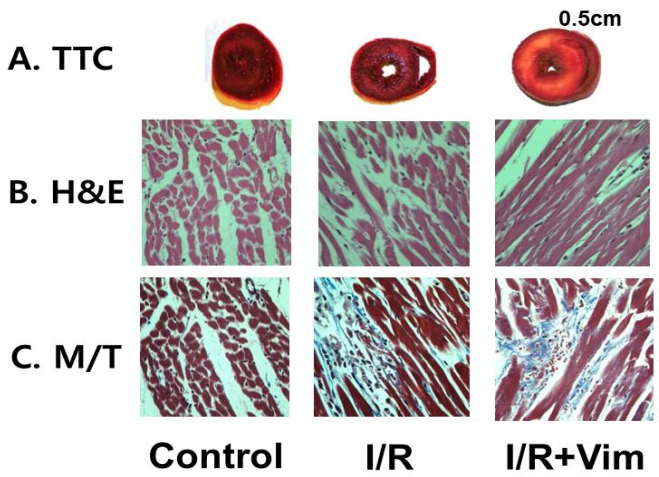

Figure 2. The assessment of the infarction among 3 groups by histology. The extent of the infarct size was assessed by histology using triphenyl tetrazolium chloride(TTC) stain (A), hematoxylin-eosin(H-E) stain (B×400), and masson trichrome(M/T) stain (C×400).

Figure 3A shows the EKG change after the I/R injury showing QRS widening and ST segment elevation. However, ST segment elevation was not improved in the I/R+Vim group. While spontaneous ventricular tachycardia (VT) or ventricular fibrillation (VF) were not observed in control rats, they were observed in 6 out of 10 (60%) I/R group and 5 out of 7 (71%) I/R+Vim group (Figure 3B). During programmed stimulation, ventricular tachyarrhythmias were induced in all survived rats of I/R and I/R+Vimentin groups (Figure 3C).

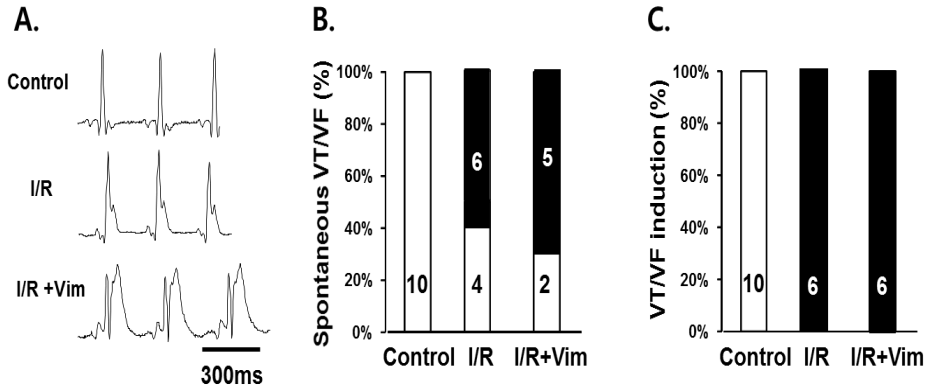

Figure 3. The comparison of arrhythmia among 3 groups. A. Compared with control, wider QRS complex was observed on electrocardiogram in the ischemia-reperfuson (I/R) and I/R+Vimentin groups. B. Spontaneous ventricular tachyarrhythmia occurred 60% in I/R and 71% in I/R+Vimentin groups. C. Ventricular tachyarrhythmia were induced in all survived rats of I/R and I/R+Vimentin groups.

2. Effects of vimentin on the Electrophysiologic Characteristics

The action potential tracings of the 3 groups are presented in Figure 4A. APD was shortened in both I/R and I/R+Vimentin group. The APD restitution were investigated from the infarct border zone in I/R and I/R+Vimentin groups compared with LV anterior wall in control group (Figure 4B). After the I/R injury (red square), the APD shortened at longer pacing CLs, but not at shorter pacing CLs, resulting in a flattening of the steepness of the APD restitution slope. A flattening of the steepness of the APD restitution slope was not changed in the I/R+Vim group (blue triangles).

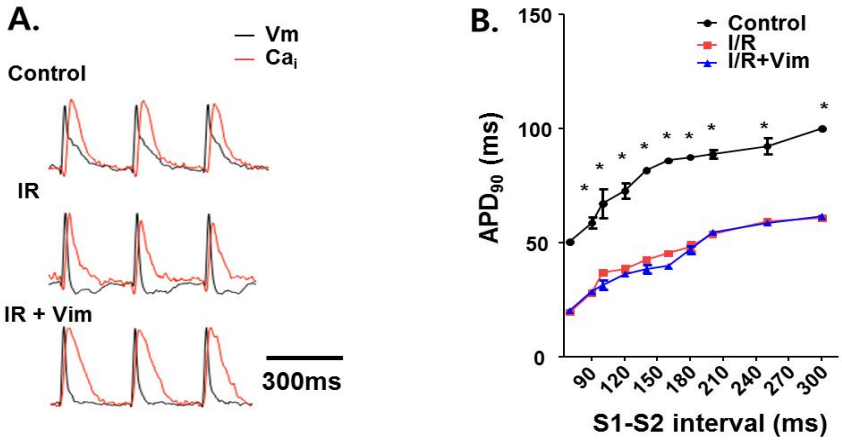

Figure 4. The action potential duration among 3 groups. A. Sample traces of V_m and Ca_i currents from the three groups exhibit an action potential duration(APD) shortening in both ischemia-reperfuson (I/R) and I/R+Vimentin groups (cycle length=300ms). B. In APD restitution kinetics curves, the steepness of APD was not changed with vimentin injection (*I/R or I/R+Vim vs. control, $P<0.01$).

Figure 5A shows activation and CV vector maps of whole rat heart from each group. The electrical conduction was not propagated to the infarct zone in both I/R and I/R+Vimentin groups. The abrupt change of CV vector was observed at the infarct zone in both I/R and I/R+Vimentin groups. The average CV was calculated from the CV vector maps at the infarct border zone, or at corresponding sites in the control. CV RK curves of 3 groups were presented in Figure 5B. CV at the pacing CL between 180 ms and 300 ms was significantly slowed in I/R group (red square) and I/R+Vimentin groups (blue triangles). The steepness of the CV RK slope was increased in both I/R and I/R+Vimentin groups. Conduction heterogeneity demonstrated similar pattern with the results of CV (Figure 5C).

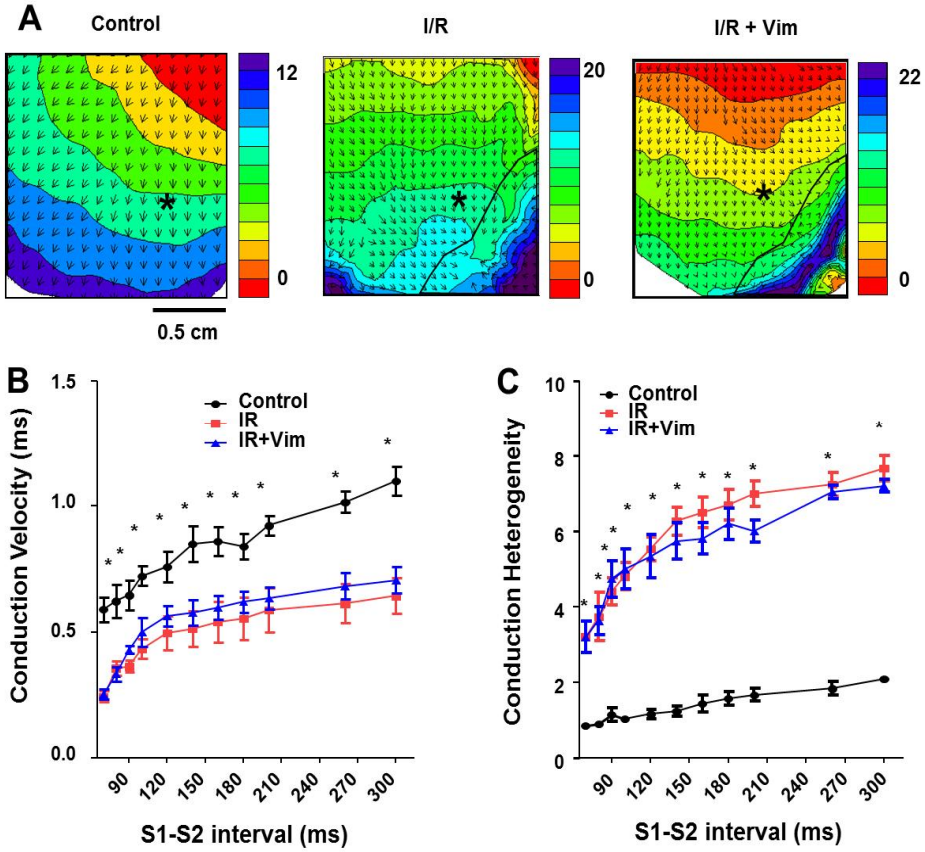

Figure 5. The conduction velocity and heterogeneity among 3 groups. A. In activation map, the activation time in ischemia-reperfuson (I/R) and I/R+Vimentin group took longer than in control. The asterisks indicate the recording sites of the V_m and Ca_i tracings in figure 4A. B. In the conduction velocity(CV) restitution kinetics curves, the steepness was not changed with vimentin injection after ischemic injury (*I/R or I/R+Vim vs. control, P<0.01). C. In the conduction heterogeneity restitution kinetics curves, the steepness was not changed with vimentin injection after ischemic injury (*I/R or I/R+Vim vs. control, P<0.01).

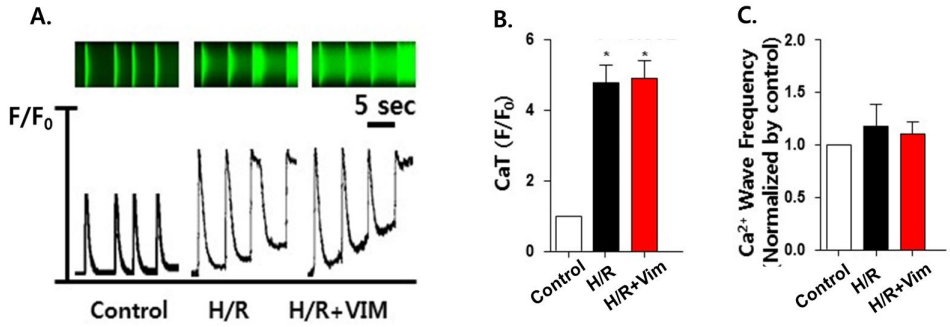

Figure 6. The calcium transient among 3 groups. A. The line scan and line plot of spontaneous Ca^{2+} transients were obtained from neonatal cardiomyocytes during the control, hypoxia- reoxygenation(H/R), and H/R+Vimentin conditions. B. The amplitudes of Ca^{2+} waves were increased in both H/R and H/R+Vimentin group (*H/R or H/R+Vimentin vs. control, P<0.01). C. The frequencies of Ca^{2+} waves were not different among 3 groups.

3. The effect of vimentin on calcium transient and contractility after the Ischemia-Reperfusion injury

Figure 6A shows representative line scan images of spontaneous Ca^{2+} transients obtained from neonatal cardiomyocytes after hypoxia and vimentin treatment. Compared with control (1.0 ± 0.0 F/F_0), the amplitude of the Ca^{2+} waves was significantly increased in both hypoxia (4.8 ± 1.0 F/F_0 vs. control, p<0.01) and hypoxia+vimentin group (4.9 ± 1.1 F/F_0 vs. control, p<0.01) (Figure 6B). The frequencies of the Ca^{2+} waves were not different among 3 groups (1.0 ± 0.0 Hz in control, 0.9 ± 0.5 Hz in I/R group and 1.1 ± 0.3 Hz in I/R+Vimentin group, p=1.0) (Figure 6C). The contractility of adult cardiomyocytes in I/R or

I/R+Vimentin groups were normalized by control group. After I/R injury, the contractility was decreased (0.5 ± 0.2 vs. control, $p<0.05$). However, after cotreatment with vimentin, the contractility was recovered nearly upto control level (1.0 ± 0.9 vs. control, p=N.S.).

IV. DISCUSSION

The main findings of this study were that the injection of vimentin one hour after I/R injury increased myocardial contractility. Second, vimentin made no change of the Ca^{2+} overloading in neonatal cardiomyocyte. Finally, vimentin did not change the vulnerability of arrhythmia, conduction velocity or conduction vector.

1. The role of vimentin on cardiac contractility

In this study, vimentin increased cardiac conduction in control cells. Vimentin is a type III IF protein that is expressed in mesenchymal cells. IF, along with tubulin-based microtubules and actin-based microfilaments, comprise the cytoskeleton. Vimentin is the major cytoskeletal component of mesenchymal cells, and is responsible for maintaining cell shape, integrity of the cytoplasm, and stabilizing cytoskeletal interactions.

The extensive T-tubular system of ventricular myocytes ensures a rapid and homogeneous increase in $[Ca^{2+}]_i$ throughout the cell. When the number of T tubules is decreased, Ca^{2+} release is more inhomogeneous.[15] This role of the T tubules is also supported by observations in neonatal ventricular myocytes, atrial myocytes, and Purkinje cells, which lack T tubules. In these cells, Ca^{2+} release during depolarization occurs first below the sarcolemmal membrane, and the increase of $[Ca^{2+}]_i$ in the center of the cell is small and delayed.[16-20]

Therefore, externally administrated vimentin might have improved integrity of the cytoplasm including T-tubule.

2. The no effect of vimentin on the calcium and arrhythmia in I/R injury model

Early reperfusion of ischemic cardiac tissue remains the most effective intervention for improving the clinical outcome following myocardial infarctions. However, abnormal increases in intracellular Ca^{2+} during myocardial reperfusion can cause cardiomyocyte death and consequent loss of the cardiac function, referred to as I/R injury. Therapeutic modulation of the Ca^{2+} handling provides some cardioprotection against the paradoxical effects of restoring the blood flow to the heart, highlighting the significance of a Ca^{2+} overload on I/R injury. In this study, externally administered vimentin made no change of the Ca^{2+} overloading of neonatal myocyte after I/R injury.

And spontaneous or induced VT/VF vulnerability were similar between I/R and I/R+Vimentin group. Electrophysiologic characteristics such as APD, CV and conduction vector also showed similar patterns whether or not vimenin was injected after I/R injury.

V. CONCLUSION

The main findings of this study were that the injection of vimentin one hour after I/R injury increased myocardial contractility. Second, vimentin made no change of the Ca^{2+} overloading in neonatal cardiomyocyte. Finally, vimentin showed no antiarrhythmic effect after I/R injury. This result suggested that vimentin might be used as a therapeutic tool to increase cardiac contractility. However, anti-arrhythmic or pro-arrhythmic effect should be further evaluated.

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ABSTRACT(IN KOREAN)

쥐의 심근에 허혈 재관류 손상이 가해졌을 때 비멘틴의 심근 수축력과 세포내 칼슘 및 심실부정맥 발생에 대한 평가

<지도교수 정 보 영>

연세대학교 대학원 의학과

문 희 선

비멘틴은 제 3형 중간 섬유로서 주요한 세포 골격 구성요소이다. 세포소기관들을 지지하고 연결하여 세포의 모양을 유지시키고 세포 소기관 간의 상호작용을 안정화시키는 것이 그 주요한 역할이다. 심실 근육세포의 T관 시스템을 통해서 Ca^{2+} 은 급격하면서도 균일하게 증가한다. 본 연구를 통해서 정주한 비멘틴이 심근세포에서도 Ca^{2+} 의 항상성 유지를 통해 수축력을 개선시키는지 그에 따른 부정맥에 대한 영향을 확인하고자 하였다.

Sprague-Dawley 쥐를 대조군, 허혈/재관류 및 비멘틴처지군 각각 10마리씩 배정하였다. 허혈/재관류 모델은 좌주간지 관상동맥을 1시간동안 결찰한 뒤 3시간동안 다시 개통하였다. 비멘틴은 허혈 1시간 뒤 125 μL을 다리정맥을 통해서 전신으로 정주하였다.

자발적인 심실부정맥이 대조군에서는 관찰되지 않았지만 허혈/재관류군에서는 60%, 비멘틴군에서는 70%에서 발생하였다. 대조군에서는 전기생리학검사로도 심실부정맥은 유도되지 않았지

만 허혈/재관류군과 비멘틴군에서는 100% 유도되었다. 대조군에 비해 허혈/재관류군 쥐의 활동전위기간은 짧았고 전도속도는 느렸으며 전도불균일도 증가하였다. 그러나 비멘틴을 처치하여도 그 양상은 허혈/재관류군과 비슷하였다. Ca^{2+} 파형의 진폭은 허혈/재관류군과 비멘틴군에서 모두 증가하였으나 빈도는 세군간에 차이가 없었다. 심근세포의 수축력은 허혈/재관류군에서는 감소하였지만 비멘틴을 처치한 군에서는 대조군 수준으로 회복되는 것을 확인하였다.

결론적으로 허혈성 손상 후에 외부에서 정주한 비멘틴은 심근세포의 수축력을 개선시키지만 Ca^{2+}의 분비에는 변화가 없었고 심실부정맥 역시 통계적으로 유의한 차이는 보이지 않았다. 이로서 심근수축력을 개선시키기 위한 목적으로 비멘틴을 고려해 볼 수 있으나 다만 부정맥과 관련해서는 추가적인 연구가 필요할 것이다.

핵심되는 말 : 비멘틴, 허혈 재관류, 부정맥, 칼슘