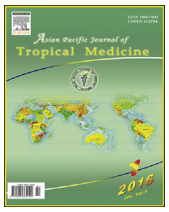
HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm



Original research http://dx.doi.org/10.1016/j.apjtm.2015.12.019

Effects of Ang II perfusion on transmural heterogeneous of Cx43 in acute myocardial ischemia reperfusion

Xiao-Pan Chen[1], Shi-Juan Lu[2], Kang Huang[2], Wei Zhang[2], Zheng-Wang Liu[1], Jiang-Hua Zhong[2*]

[1]*Hainan Medical University Affiliated Hospital, Haikou 570102, China*

[2]*Central South University Xiangya School of Medicine Affiliated Haikou Hospital, Haikou People's Hospital, Haikou 570208, China*

ARTICLE INFO

Article history:
Received 15 Oct 2015
Received in revised form 20 Nov 2015
Accepted 3 Dec 2015
Available online 18 Dec 2015

Keywords:
Acute myocardial ischemia reperfusion
Dispersion of repolarization
Cx43
Angiotensin II

ABSTRACT

Objective: To observe the effects of angiotensin II(Ang II) perfusion on transmural heterogeneity of Cx43 expression in the rabbit model with acute myocardial ischemia reperfusion (MIR), and investigate the role of rennin-angiotensin system in malignant ventricular arrhythmia induced by MIR.

Methods: Twenty rabbits were randomly divided into MIR group (n = 10) and Ang II group (n = 10). MIR model was produced with traditional ligation and opening of the anterior descending coronary artery in all animal. The hearts *in vitro* in the MIR group and the Ang II group were perfused with simply improved Tyrode's solution and containing Ang II Tyrode's solution respectively. 90% monophasic action potential repolarization duration, transmural dispersion of repolarization, Cx43 protein (Cx43-pro) and mRNA (Cx43-Cq) expression in subepicardial, midmyocardial and subendocardial myocardium were measured in both groups. The greatest differences of Cx43-pro and Cx43-Cq among three myocardial layers were calculated and shown with ΔCx43-pro and ΔCx43-Cq respectively.

Results: After Ang II perfusion, 90% monophasic action potential repolarization duration among three myocardial layer were significantly prolonged ($P < 0.05$ and $P < 0.01$), and transmural dispersion of repolarization also significantly increased compared with the MIR group ($P < 0.05$). Compare with the MIR group, three myocardial Cx43-pro and Cx43-Cq expression in the Ang II group were significantly decreased ($P < 0.05$ and $P < 0.01$), but ΔCx43-pro and ΔCx43-Cq were significant increased.

Conclusions: Renin-angiotensin system increases transmural heterogeneity of Cx43 expression in the rabbit model with MIR by Ang II, and enlarge transmural dispersion of repolarization among three myocardial layers of left ventricular which induces malignant ventricular arrhythmia.

1. Introduction

Acute myocardial ischemia reperfusion easily causes malignant ventricular arrhythmia (MVA) and sudden cardiac death, whose mechanism is in need of further investigation. Besides, angiotensin II (Ang II), the main element of rennin-angiotensin systems (RAS), is actively involved in the cause and development of cardiovascular diseases, such as hypertension, heart failure and myocardial infarction. According to recent researches, Ang II can influence Cx43 remodeling and change myocardial electrophysiology parameter and it is closely related to MVA and sudden death [1,2], which indicates that Ang II-mediated gap reconstruction caused abnormal electrophysiology.

We observe effects of Ang II perfusion on myocardial ischemia reperfusion (MIR) rabbit model transmural dispersion of repolarization and expression heterogeneity of Cx43, and discuss occurrence mechanism of MVA in MIR and the therapeutic target through making MIR rabbit model in our research.

*Corresponding author: Jiang-Hua Zhong, Central South University Xiangya School of Medicine Affiliated Haikou Hospital, Haikou People's Hospital, Haikou 570208, Hainan, China.
E-mail: zhong3882@163.com

Peer review under responsibility of Hainan Medical College.
Foundation project: It is supported by National Natural Science Foundation of China (NO. 81160024) and Natural Science Foundation of Hainan Province (NO. 814371).

2. Materials and methods

2.1. Animal grouping and model making

Twenty healthy rabbits, provided by Laboratory Animal Center of Hainan Medical University, weighting (2.0–3.0) kg, of either sex, were divided into MIR control group (n = 10) and Ang Ⅱ reperfusion group (n = 10). Both groups of rabbits were weighted and anaesthetized with urethane, and their right common carotid arteries were separated. A plastic catheter was incubated through right common carotid arteries to measure mean arterial pressure. Their tracheas were incubated and connected to artificial respirators. The chests were open along the median line of breastbone. The pericardia were cut open to fully expose left anterior free walls of hearts and make pericardial cradles. Left ventricular branch of the coronary artery was separated and the anterior descending artery was threaded and ligated at 0.3 cm away from the lower margin of left atrial appendage. Observe the electrocardiogram and take ST-segment elevation >0.5 mV in two adjacent leads as the indicator of myocardial ischemia. After 20 min observation, ligature was cut and myocardium was reperfused. The standard of reperfusion damage formation was that after reperfusion ST segment was decreased >50%, and R wave amplitude was decreased with occurrence of arrhythmia and Q wave. After models were made, MIR control group was perfused with improved Tyrode's solution, while Ang Ⅱ perfusion group was perfused with Tyrode's solution with 1 μmoL Ang Ⅱ.

2.2. Experiment solution preparation

Ingredients improved Tyrode's solution for isolated heart perfusion (mmoL/L): NaCl 115, KCl 5.4, $MgCl_2$ 1, $CaCl_2$ 1.8, NaH_2PO_4 1, HEPES 5, glucose 10, adjusting pH value to 7.4 [3]. Prepare eppendorf tubes containing buffer solution with pH value of 8, 1 mmoL/L $NaHCO_3$, 5 mmoL/L EDTA, 1 mmoL/L EGTA, 1 μmoL/L leupeptin, 1 μmoL/L pepstatin, 100 nmoL/L aprotinin, 1 mmoL/L benzamidine, 1 mmoL/L iodoacetamide and 1 mmoL/L PMSF [4].

2.3. In vitro cardiac electrophysiology experiment

We referred to previous experiment method and made electrophysiological experiments as follow:

2.3.1. Pre-experiment preparation

Hearts were quickly cut down and put in 4 °C Tyrode's solution to arrest. Washed by precool normal saline, the whole hearts were weighted. After aortic cannula, the hearts were perfused with Langendorff [pure oxygen saturation, constant temperature of (36.5–37.5) °C, constant pressure of (8.52–8.78) kPa].

2.3.2. Electrophysiological experiment

Pacemaker electrode is consisted of two pin electrodes. The distance between the two electrodes is 0.5 cm, pricking into the position where the heartbeat was strongest. Reference electrodes were fixed on the root of aorta. Simplified electrodes of endocardium, epicardium and midmyocardium were placed in the needles of syringes respectively. The needles were fixed 10 mm away from apical and pricked in the position 5 mm away from interventricular septum. Endocardium electrodes were fixed in the subendocardial myocardium. Epicardium electrodes were fixed 3.0 mm away from lateral epicardium of left ventricular. Midmyocardium electrodes were fixed to make pinpoint of electrode 3.0 mm away from epicardial surface. Simplified electrodes of monophasic action potentials of three myocardial layers were fixed and connected to biological signal acquisition and processing system and the parameter was set as filter (500–1 000) Hz. Along the junction of free walls of right atrium and crista terminalis, sinoatrial node was cut by scissors, and slow nodal rhythm appeared. Cardiac electrophysiology stimulation was fixed at the wall of right ventricle and cardiac pacing was performed with pacing cycle length 1 000 ms. All rabbits were perfused by normal Tyrode's solution for 20 min, and then steady monophasic action potentials of three myocardial layers were steadily recorded.

2.3.3. Post–experiment process

When experiments were finished, hearts were cut down and their wet weights were measured. Cardiac tissues of reperfused zone were obtained to produce pathological specimen.

2.4. Protein extracted from three myocardial layers of left ventricular

After electrophysiology experiment, free walls of left ventricular from rabbits were cut down and stored in liquid nitrogen. By the way of fast frozen section, from the surface of epicardium to endocadium, epicardial cardiomyocyte of left ventricular were obtained to make 10 serial section of 20 μm (altogether 200 μm). In the same way, from the surface of endocadium to epicardium, endocardial cardiomyocyte of left ventricular were obtained to make 10 serial section of 20 μm (altogether 200 μm). Midmyocardial cardiomyocyte, 3 mm away from the surface of epicardium, were obtained to make 10 serial section of 20 μm (altogether 200 μm) [3]. Cardiomyocyte of three layers were stored in prepared eppendorf tubes respectively.

2.5. Cx43 expression detected by Western-blot

Samples in eppendorf tubes were homogenated, extracted protein, loaded, and turned into PVDF membrane through SDS polyacrylamine gel electrophoresis. PVDF membrane were sealed in 5% TBST skim milk for 2 h in indoor temperature. Cx43 monoclonal antibody (Zymed 35-5000) was dropped in and kept over night at 4 °C. Samples were added horseradish peroxidase labeled second antibody (Novogene company), and incubated for 2 h at indoor temperature. Colored by enhanced chemiluminescence, samples were developed on X-ray films. Protein signal strip image was obtained through gel image analysis system to calculate target Cx43 and optical density of internal reference protein GAPDH, taking their ratio as the expression level of Cx43.

2.6. Expression of Cx43 mRNA detected by RT-qPCR

Frozen tissue samples of left ventricular of both groups in eppendorf tubes were ground into powder, from which total RNA was extracted. cDNA was compound by RevertAid First Strand reverse transcriptase kits, and amplified by qPCR, taking *GAPDH* as internal reference. Upstream primer:

5′-GAGGTGGCCTTCTTGCTGAT-3′, downstream primer: 5′-GTTTTCTCAGTGGGGCGAGA-3′, The length of estimated amplified fragment was 122 bp. *GAPDH* upstream primer: 5′-AGAGCACCAGAGGAGGACGA-3′, downstream primer: 5′-TGGGATGGAAACTGTGAAGAGG-3′, the length of estimated amplified fragment was 104 bp. Reverse transcription parameter: 42 °C 60 min, 70 °C 5 min qPCR reaction parameter: 50 °C 3 min, 95 °C 15 min, 95 °C 10 s, 60 °C 20 s, 72 °C 30 s, repeated 40 cycles. During qPCR amplification, fluorescence was collected. When reaction was finished, cycle threshold detected by all fluorescence signals of samples during qPCR was analyzed and calculated. Cycle quantification of *Cx43* mRNA of samples was calculated by $2^{-\Delta\Delta Ct}$ method to present relative expression level.

2.7. Observed indicator

(1) APD parameter: ① 90% repolarization of action potential duration (APD_{90}): APD from phase 0, repolarization, to amplitude of 90%. ② Transmural dispersion of repolarization (TDR): The difference between the longest APD_{90} and the shortest APD_{90} among three myocardial layers. (2) Cx43 protein: Expression levels of Cx43 of every myocardial layer of left ventricular. (3) ΔCx43-pro: The difference of Max Cx43-pro and Min Cx43-pro among three myocardial layers. (4) Cx43-Cq: Expression levels of Cx43 mRNA protein of every myocardial layer of left ventricular. (5) ΔCx43-Cq: Greatest difference of Max and Min Cx43-Cq value among three myocardial layers.

2.8. Statistical method

SPSS 13.0 statistical software was used in statistic and detection. All experimental materials were randomly designed. One-way ANOVA was taken to performed significance analysis of difference among groups. SNK was used to test multiple comparison of variance homogeneity among groups. Variance heterogeneity was tested by Tamhane. If $P < 0.05$, it has statistical meaning.

3. Results

3.1. Fundamental changes

Mean arterial pressure, heart/body weight, left ventricular weight of two groups did not differ significantly ($P > 0.05$). However, in pathological section examination, disordered myocardial hypertrophy of left ventricular was observed, conforming to pathological manifestation of MIR.

3.2. Comparison of electrophysiological parameter of left ventricular

Compared with MIR control group, perfused by Ang Ⅱ, APD_{90} of three myocardial layers were all further prolonged ($P < 0.05$ and $P < 0.01$), and TRD was increased ($P < 0.05$), which indicated that Ang Ⅱ perfusion could enlarge transmural repolarization heterogeneity of MIR (Table 1).

Table 1
Electrophysiological parameter comparison between two groups of rabbits (mean ± SD).

Observed indicator	MIR control group	Ang Ⅱ perfusion group
Endo-APD_{90} (ms)	229.60 ± 18.12	248.90 ± 22.43[a]
Mid-APD_{90} (ms)	277.80 ± 20.12	304.40 ± 20.36[b]
Epi-APD_{90} (ms)	227.70 ± 19.94	246.80 ± 17.16[a]
TDR (ms)	58.30 ± 10.63	68.40 ± 11.70[a]

Compared with MIR control group, [a]$P < 0.05$, [b]$P < 0.01$.

3.3. Comparison of left ventricular myocardial transmural Cx43 expression

Perfused by Ang Ⅱ, all Cx43 expression of three myocardial layers decreased than that of MIR control group ($P < 0.05$ and $P < 0.01$), and ΔCx43-pro and ΔCx43-Cq increased significantly ($P < 0.05$), which indicated that Ang Ⅱ perfusion not only weakened MIR three myocardial layers Cx43 expression, but also increased heterogeneity of transmural Cx43 expression (Table 2).

4. Discussion

Acute myocardial ischemia (AMI) is a clinical incident greatly threatening patients' lives. The key to treatment of AMI is to open infarcted vessels as soon as possible. AMI reperfusion often accompanies with myocardial damage. Arrhythmia is the most common form of clinical reperfusion damage. Any kind of myocardial reperfusion will cause reperfusion arrhythmia with rate of 50%–80%. Arrhythmia, especially ventricular arrhythmia, is one of the main causes of exacerbation and sudden cardiac death by changing the hemodynamic of patients. However, at present, the physiological mechanism of easy-happening MVA is not totally clear.

Ang Ⅱ is the main ingredient of RAS, widely involved in the cause and development of cardiovascular diseases. According to recent researches, Ang Ⅱ also influence myocardial electrophysiology parameter and participate in the occurrence of MVA [4,5]. RAS antagonist, blocking Ang, can effectively reduce organic heart disease and tachyarrhythmia [6,7]. Further electrophysiological studies found that RAS antagonist can improve ventricular repolarization dispersion, inhibit the exchange of Na^+/H^+ and Na^+/Ca^{2+}, and block L-type Ca^{2+} channel [8]. However, specific mechanism of RAS's role in malignant arrhythmia is not clear. Our results showed that, compared with MIR control group, APD_{90} among three myocardial layers were significantly prolonged ($P < 0.05$), and TDR also significantly increased compared with the MIR group ($P < 0.05$). These indicated that, as Ang Ⅱ perfusion prolonged APD_{90} among three myocardial layers, it further worsened transmural dispersion of ventricular repolarization. This may be the electrophysiologic mechanism of RAS's participation in MIR and MVA.

Ang Ⅱ also got involved in the regulation of Cx by reducing Cx43 and slowing down conduction. Effect of Ang Ⅱ on expression of ventricular myocardium gap junction may be the

Table 2
Comparison of left ventricular myocardial transmural Cx43 expression.

Observed indicator	MIR control group	Ang Ⅱ perfusion group
Cx43-pro-Endo	0.46 ± 0.07	0.39 ± 0.09[a]
Cx43-pro-Mid	0.33 ± 0.06	0.22 ± 0.05[b]
Cx43-pro-Epi	0.47 ± 0.07	0.40 ± 0.07[a]
ΔCx43-pro	0.17 ± 0.04	0.22 ± 0.06[a]
Cx43-Cq-Endo	20.35 ± 3.02	16.04 ± 2.65[b]
Cx43-Cq-Mid	16.88 ± 2.26	12.46 ± 2.37[b]
Cx43-Cq-Epi	20.12 ± 3.17	16.46 ± 2.88[a]
ΔCx43-Cq	3.73 ± 1.06	4.47 ± 0.69[a]

Compared with MIR control group, [a]$P < 0.05$, [b]$P < 0.01$.

important mechanism to cause ventricular arrhythmia [9]. According to recent studies [9], angiotensin converting enzyme 2 can turn Ang Ⅱ into Angl-7 through enzymatic hydrolysis, reduce Ang Ⅱ, and trigger reconstruction of ventricular protein GJ, especially Cx43, which is closely related to arrhythmia [10]. This also indicated that Ang Ⅱ-mediated gap reconstruction caused abnormal electrophysiology. Our results further showed that, compared with MIR control group, in Ang Ⅱ perfusion group expression of Cx43 and mRNA of endocardium, epicardium and midmyocardium decreased significantly ($P < 0.05$), while ΔCx43 further increased, which indicated that over activation of Ang Ⅱ increase the transmural expression heterogeneity of Cx43 in MIR. This may be the important molecule mechanism of RAS's involvement in MIR MVA.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Yasuno S, Kuwahara K, Kinoshita H, Yamada C, Nakagawa Y, Usami S, et al. Angiotensin Ⅱ type 1a receptor signalling directly contributes to the increased arrhythmogenicity in cardiac hypertrophy. *Br J Pharmacol* 2013; **170**(7): 1384-1395.

[2] Sovari AA, Rutledge CA, Jeong EM, Dolmatova E, Arasu D, Liu H, et al. Mitochondria oxidative stress, connexin43 remodeling, and sudden arrhythmic death. *Circ Arrhythm Electrophysiol* 2013; **6**(3): 623-631.

[3] Yamada KA, Kanter EM, Green KG, Saffitz JE. Transmural distribution of connexins in rodent hearts. *J Cardiovasc Electrophysiol* 2004; **15**(6): 710-714.

[4] Sovari AA, Iravanian S, Dolmatova E, Jiao Z, Liu H, Zandieh S, et al. Inhibition of c-Src tyrosine kinase prevents angiotensin Ⅱ-mediated connexin-43 remodeling and sudden cardiac death. *J Am Coll Cardiol* 2011; **58**(22): 2332-2339.

[5] Wang YH, Shi CX, Dong F, Sheng JW, Xu YF. Inhibition of the rapid component of the delayed rectifier potassium current in ventricular myocytes by angiotensin Ⅱ via the AT1 receptor. *Br J Pharmacol* 2008; **154**(2): 429-439.

[6] Zhang H, Zhong H, Everett TH IV, Wilson E, Chang R, Zeng D, et al. Blockade of A_{2B} adenosine receptor reduces left ventricular dysfunction and ventricular arrhythmias 1 week after myocardial infarction in the rat model. *Heart Rhythm* 2014; **11**(1): 101-109.

[7] Jiao KL, Li YG, Zhang PP, Chen RH, Yu Y. Effects of valsartan on ventricular arrhythmia induced by programmed electrical stimulation in rats with myocardial infarction. *J Cell Mol Med* 2012; **16**(6): 1342-1351.

[8] Yan QL, Hua W, Yu LZ. The functional changes in L-type Ca^{2+} channel of hypertrophied cardiomyocytes in neonatal rats induced by angiotensin Ⅱ. *Chin J Appl Physiol* 2013; **29**(1): 91-95.

[9] Matsushita N, Hirose M, Sanbe A, Kondo Y, Irie Y, Taira E. Nicorandil improves electrical remodelling, leading to the prevention of electrically induced ventricular tachyarrhythmia in a mouse model of desmin-related cardiomyopathy. *Clin Exp Pharmacol Physiol* 2014; **41**(1): 89-97.

[10] Fan JQ, Zou LL, Cui K, Woo K, Du HA, Chen SJ, et al. Atrial overexpression of angiotensin-converting enzyme 2 improves the canine rapid atrial pacing-induced structural and electrical remodeling. Fan, ACE2 improves atrial substrate remodeling. *Basic Res Cardiol* 2015; **110**(4): 45.