Journal of Hainan Medical University

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Correlation between myocardial ischemia−reperfusion−induced monophasic action potential amplitude change and myocardial damage

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ARTICLE INFO

Article history:
Received 28 Aug 2017
Received in revised form 3 Sep 2017
Accepted 9 Sep 2017
Available online 14 Sep 2017

Keywords:
Myocardial ischemia-reperfusion
Monophasic action potential
Oxidative stress
Apoptosis

ABSTRACT

Objective: To study the correlation between myocardial ischemia-reperfusion-induced monophasic action potential amplitude (MAPA) change and myocardial damage. **Methods:** New Zealand rabbits were selected as experimental animals and randomly divided into control group and ischemia-reperfusion group (I/R group), myocardial ischemia-reperfusion injury models were established, then the heart was separated and the MAPA of myocardial intima layer, media layer and outer layer were determined in Langendorff perfusion system; serum samples and myocardial tissue were collected to determine the contents of myocardial injury molecules. **Results:** MAPA levels of myocardial intima layer, media layer and outer layer of I/R group were significantly lower than those of control group; CK-MB, cTnI, cTnT and MDA contents in serum as well as Bax, Caspase-3 and Caspase-9 mRNA expression in myocardial tissue of I/R group were significantly higher than those of control group and negatively correlated with MAPA levels of myocardial intima layer, media layer and outer layer while SOD, GSH-Px and HO-1 contents in serum as well as Bcl-2 and Bcl-xL mRNA expression in myocardial tissue were significantly lower than those of control group and positively correlated with MAPA levels of myocardial intima layer, media layer and outer layer. **Conclusion:** Myocardial ischemia - reperfusion can induce the decrease of MAPA and is closely related to myocardial oxidative stress injury and apoptosis.

1. Introduction

Acute myocardial infarction is a common disease in the cardiovascular system. The progress of interventional, thrombolytic and other reperfusion therapies in recent years has greatly improved patients' prognosis. However, affected by ischemia reperfusion, myocardial cell injury is increased after patients with myocardial infarction receive interventional or thrombolytic therapy[1,2], and the risk of arrhythmia increases[3]. Oxidative stress and apoptosis are closely related to the ischemia reperfusion injury of myocardial cells, but the mechanism of arrhythmia after ischemia reperfusion has not been clear. Monophanic action potential (MAP) is the action potential of the local in vivo heart beat tissue, which can reflect the integrated electrocardial vector of multiple cells in the local heart tissues. In the case of myocardial ischemia injury and ischemia reperfusion injury, the damage and destruction of myocardial cells can cause the change in the action potential amplitude and repolarization process. Monophasic action potential amplitude (MAPA) is a common electrophysiological index to evaluate monophasic action potentials, the correlation between myocardial ischemia-reperfusion-induced monophasic action potential amplitude change and myocardial damage we specifically analyzed in the following study.

2. Experimental materials and methods

2.1 Experimental materials

A total of 16 male New Zealand rabbits with body mass of 2.0-3.0 kg were selected as experimental animals and purchased from

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Fund Project: Project of Sichuan Provincial Medical Association No.: Q14049.

Shandong Michel Biological Products Co., Ltd., and randomly divided into I/R group and control group. The enzyme-linked immunosorbent assay kits and radioimmunoprecipitation kits were purchased in Shanghai Westang Biological Company, and fluorescence quantitative PCR kits were purchased from Beijing CWBIO Company.

2.2 Experimental methods

2.2.1 Model establishment

I/R group of animals were established into myocardial ischemia reperfusion models according to the following method: 10% chloral hydrate 400 mg/kg was injected via ear vein, endotracheal intubation and assisted ventilator breathing were conducted after anesthesia, about 10 cm long incision was made in the left sternum, the three ribs on xiphoid process were separated and cut short, tissue was separated to expose the heart, the left aurcle and and the start of coronary artery were separated carefully, the myocardial blood flow was blocked for 40 min after threading and ligation, the knot was loosened for 120 min of reperfusion, and the heart was taken for subsequent experiments. Control group received anesthesia and coronary artery separation in the same way as those of I/R group, threading was conducted without ligation, and the heart was taken directly after 160 min for subsequent experiments.

2.3 MAPA detection

After model establishment, the heart was quickly taken out and placed in 4 ℃ K-H solution, the tissue around the aorta was trimmed to free the aorta, the extravasated blood was cleared for aortic cannula, then the heart was fixed in the Langendorff perfusion device for 37 ℃ constant temperature and 8.82 kPa constant pressure perfusion in K-H solution, monophasic action potential combined electrodes were placed after heart re-beating, outer layer electrode was placed on the surface of the heart, the intima layer and media layer were placed 2 mm apart respectively, and action potential was recorded by software and then analyzed to get MAPA.

2.4 Detection of myodardial damage indexes in serum

120 min after reperfusion, 3-5 mL of peripheral blood was collected from two groups of animals, let stand for coagulation and then centrifuged for 20 min to separate the upper serum, enzyme-linked immunosorbent assay kit was used to detect the contents of CK-MB, cTnI and cTnT, and radioimmunoprecipitation kits were used to detect the contents of MDA, SOD, GSH-Px and HO-1.

2.5 Detection of myodardial damage indexes in myocardium

After MAPA detection, adequate amount of myocardial tissue was collected and added in Trizol lysate to extract the RNA in the tissue, reverse transcription kit was used to synthesize RNA into cDNA by reverse transcription, fluorescence quantitative PCR kit was used to amplify Bax, Bcl-2, Bcl-xL, Caspase-3 and Caspase-9, and the relative expression of mRNA was calculated according to amplification curve.

2.6 Statistical methods

SPSS 20.0 software was used to input data, the differences in data between two groups were by t test, and $P<0.05$ indicated statistical significance in differences.

3. Results

3.1 MAPA of myocardial intima layer, media layer and outer layer

Analysis of MAPA levels of myocardial intima layer, media layer and outer layer of two groups of animals was as follows: MAPA levels of myocardial intima layer, media layer and outer layer of I/R group were significantly lower than those of control group. Differences in MAPA levels of myocardial intima layer, media layer and outer layer were statistically significant between I/R group and control group ($P<0.05$). Data were shown in Table 1.

Table 1.

Comparison of MAPA of myocardial intima layer, media layer and outer layer between the two groups (mV).

Groups	*n*	Intima layer	Media layer	Outer layer
I/R group	8	9.22±1.05	8.36±0.94	8.83±1.07
Control group	8	14.52±1.89	14.88±1.64	15.61±2.24
T		8.827	8.092	9.782
P		<0.05	<0.05	<0.05

3.2 Myocardial damage marker contents in serum

Analysis of myocardial damage markers CK-MB, cTnI and cTnT contents in serum between two groups of animals was as follows: serum CK-MB, cTnI and cTnT contents of I/R group were significantly higher than those of control group. Differences in serum CK-MB, cTnI and cTnT contents were statistically significant between I/R group and control group ($P<0.05$). Pearson correlation analysis showed that MAPA levels of I/R myocardial intima layer, media layer and outer layer were negatively correlated with serum CK-MB, cTnI and cTnT contents.

Table 2.

Comparison of myocardial damage markers in serum between the two groups.

Groups	*n*	CK-MB	cTnI	cTnT
I/R group	8	2.03±0.28	4.85±0.62	1.88±0.23
Control group	8	0.42±0.07	1.03±0.15	0.48±0.07
T		31.822	26.866	23.219
P		<0.05	<0.05	<0.05

3.3 Oxidative stress index contents in serum

Analysis of oxidative stress indexes MDA, SOD, GSH-Px and HO-1 contents in serum between two groups of animals was as follows: serum MDA content of I/R group was significantly higher than that of control group while SOD, GSH-Px and HO-1 contents were significantly lower than those of control group. Differences in serum MDA, SOD, GSH-Px and HO-1 contents were statistically significant between I/R group and control group (P<0.05). Pearson correlation analysis showed that MAPA levels of I/R myocardial intima layer, media layer and outer layer were negatively correlated with serum MDA content and positively correlated with serum SOD, GSH-Px and HO-1 contents.

Table 3.

Comparison of oxidative stress indexes in serum between the two groups.

Groups	n	MDA	SOD	GSH-Px	HO-1
I/R group	8	13.29±1.88	52.92±7.71	45.41±5.64	24.52±3.58
Control group	8	5.62±0.74	114.62±13.28	102.34±12.51	70.61±8.52
T		13.928	10.082	12.586	15.592
P		<0.05	<0.05	<0.05	<0.05

3.4 Apoptosis molecule expression in myocardial tissue

Analysis of Bax, Bcl-2, Bcl-xL, Caspase-3 and Caspase-9 expression in myocardial tissue between two groups of animals was as follows: Bax, Caspase-3 and Caspase-9 mRNA expression in myocardial tissue of I/R group were significantly higher than those of control group while Bcl-2 and Bcl-xL mRNA exression were significantly lower than those of control group. Differences in Bax, Bcl-2, Bcl-xL, Caspase-3 and Caspase-9 expression in myocardial tissue were statistically significant between I/R group and control group (P<0.05). Pearson correlation analysis showed that MAPA levels of I/R myocardial intima layer, media layer and outer layer were negatively correlated with Bax, Caspase-3 and Caspase-9 mRNA expression in myocardial tissue and positively correlated with Bcl-2 and Bcl-xL mRNA expression in myocardial tissue.

4. Discussion

Myocardial ischemia reperfusion is an important pathophysiologic change after myocardial infarction reperfusion therapy, and also an important factor causing the increased risk of myocardial injury aggravation and arrhythmia after reperfusion treatment[4,5]. Increased formation of oxygen free radicals, activated oxidative stress reaction and initiated apoptosis pathway are thought to be closely related to the occurrence of myocardial ischemia-reperfusion injury, but the pathogenesis of arrhythmia caused by ischemia reperfusion is not yet clear. Monophasic action potential (MAP) is the integrated electrocardial vector used for reflecting multiple cells within local heart tissue, and using in vitro heart model to record MAP can intuitively and stably reflect the overall depolarization and repolarization process of myocardial tissue[6]. In the case of myocardial cell ischemia hypoxia and ischemia reperfusion, cell membrane ATP channels open and cause increased potassium efflux and decreased calcium influx, causing the reduction of action potential amplitude and the shortening of action potential duration[7,8]. In order to define the changes of cardiac electrophysiological characteristics in the process of myocardial ischemia reperfusion, MAPA of ischemia-reperfusion myocardial tissue was analyzed in the study, and the results showed that MAPA levels of myocardial intima layer, media layer and outer layer of I/R group were significantly lower than those of control group. This indicates that ischemia reperfusion can cause changes in myocardial electrophysiological characteristics and decrease the MAP amplitude of myocardial tissue.

MAPA can reflect the the integrated electrocardial vector of multiple cells within the measured tissue, and the myocardial cell damage and necrosis during myocardial tissue ischemia reperfusion will affect cell action potential and be manifested as the changes in the MAP. In order to clarify the relationship between the extent of myocardial injury and the change of MAPA, the contents of myocardial injury markers in the serum of experimental animals were first analyzed in the study. CK-MB, cTnI and cTnT are the common markers to evaluate myocardial cell damage degree, CK-MB is involved in the catalysis of glycolysis process within myocardial cells[9,10], and the cTnI and cTnT are involved in the formation of the myocardial cytoskeleton structure[11,12]. In the study, analysis of the changes in the contents of these myocardial injury markers in serum after myocardial ischemia reperfusion showed that serum CK-MB, cTnI and cTnT contents of I/R group were significantly higher than those of control group. This indicates that ischemia-reperfusion can cause myocardial cell injury and rupture, which can cause the release of marker molecules from myocardial cells into the blood circulation. Further analysis of the correlation between the degree of myocardial injury and the change of MAPA showed that MAPA levels of I/R myocardial intima layer, media layer and outer layer were negatively correlated with serum CK-MB, cTnI and cTnT contents. This confirms that the changes in MAPA caused by ischemia reperfusion are closely related to the damage and destruction of myocardial cells.

Increased oxygen free radical generation and activated oxidative stress response are the important pathological changes in myocardial ischemia reperfusion process. Hypoxia conditions will cause

Table 4.

Comparison of apoptosis molecules in myocardial tissue between the two groups.

Groups	n	Bax	Bcl-2	Bcl-xL	Caspase-3	Caspase-9
I/R group	8	2.84±0.39	0.32±0.05	0.38±0.06	3.47±0.49	3.11±0.42
Control group	8	1.03±0.14	1.02±0.14	0.97±0.12	1.05±0.11	1.01±0.15
T		17.498	20.393	15.686	24.218	19.985
P		<0.05	<0.05	<0.05	<0.05	<0.05

mitochondria injury of myocardial cells, and blood perfusion recovery after reperfusion can increase the oxygen content of local tissue and cause loss of oxidation - phosphorylation decoupling in the respiratory chain, thereby increasing the production of oxygen free radicals. The continuously produced oxygen free radicals in myocardial tissues will directly destroy the cellular structure, cause myocardial damage and increase the generation of oxidative product MDA[13,14]. At the same time, the massively generated oxygen free radicals will continuously consume the antioxidant enzymes such as SOD, GSH-Px and HO-1, which will weaken the antioxidant capacity of the local tissues. In the study, the analysis of the changes in the contents of these oxidative stress molecules in serum after myocardial ischemia reperfusion showed that serum MDA content of I/R group was significantly higher than that of control group while SOD, GSH-Px and HO-1 contents were significantly lower than those of control group. This indicates that the massive generation of oxygen free radicals and the continuous consumption of antioxidant enzymes are closely related to myocardial ischemia reperfusion injury. Further analysis of the correlation between myocardial oxidative stress damage degree and MAPA change showed that MAPA levels of I/R myocardial intima layer, media layer and outer layer were negatively correlated with serum MDA content and positively correlated with serum SOD, GSH-Px and HO-1 contents. This confirms that the changes in MAPA caused by ischemia reperfusion are closely related to oxidative stress injury of myocardial cells due to the massive generated oxygen free radicals.

The accumulation of oxygen free radicals in local myocardium can not only induce cell damage by oxidative stress, but also act on the mitochondria and activate the mitochondrial pathway of apoptosis[15]. The Bcl-2 family is the important regulating factor of mitochondrial pathways of apoptosis, the Bcl-2 and Bcl-xL have antiapoptotic activity, Bax has pro-apoptotic activity, and they can affect the downstream Caspase-3 and Caspase-9 activation to regulate apoptosis[16]. In the study, analysis of the changes in the expression of these apoptosis molecules in local tissue after myocardial ischemia reperfusion showed that Bax, Caspase-3 and Caspase-9 mRNA expression in myocardial tissue of I/R group were significantly higher than those of control group while Bcl-2 and Bcl-xL mRNA exression were significantly lower than those of control group. This indicates that excessive apoptosis is closely related to myocardial ischemia reperfusion injury. Further analysis of the correlation between myocardial cell apoptosis and MAPA change showed that MAPA levels of I/R myocardial intima layer, media layer and outer layer were negatively correlated with Bax, Caspase-3 and Caspase-9 mRNA expression in myocardial tissue and positively correlated with Bcl-2 and Bcl-xL mRNA expression in myocardial tissue. This confirms that the changes in MAPA caused by ischemia reperfusion are closely related to excessive apoptosis.

In this study, the changes in electrophysiological characteristics of local tissue in the process of myocardial ischemia reperfusion were mainly analyzed, and the results show that myocardial ischemia-reperfusion could cause MAPA to reduce and is closely related to the oxidative stress reaction activation, excessive apoptosis and MAPA change.

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