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Hypercholesterolemia aggravates myocardial ischemia reperfusion injury via activating endoplasmic reticulum stress-mediated apoptosis

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ABSTRACT

The effect of hypercholesterolemia on myocardial ischemia reperfusion injury (MIRI) is in controversy and the underlying mechanism is still not well understood. In the present study, we firstly detected the effects of hypercholesterolemia on MIRI and the role of endoplasmic reticulum (ER) stress-mediated apoptosis pathway in this process. The infarct size was determined by TTC staining, and apoptosis was measured by the TUNEL method. The marker proteins of ER stress response and ER stress-mediated apoptosis pathway were detected by Western blot. The results showed that high cholesterol diet-induced hypercholesterolemia significantly increased the myocardial infarct size, the release of myocardium enzyme and the ratio of apoptosis, but did not affect the recovery of cardiac function. Moreover, hypercholesterolemia also remarkably up-regulated the expressions of ER stress markers (glucose-regulated protein 78 and calreticulin) and critical molecules in ER stress-mediated apoptosis pathway (CHOP, caspase 12, phospho-JNK). In conclusion, our study demonstrated that hypercholesterolemia enhanced myocardial vulnerability/sensitivity to ischemia reperfusion injury involved in aggravation the ER stress and activation of ER stress-mediated apoptosis pathway and it gave us a new insight into the underlying mechanisms associated with hypercholesterolemia-induced exaggerated MIRI and also provided a novel target for preventing MIRI in the presence of hypercholesterolemia.

1. Introduction

Ischemic heart disease, especially acute myocardial infarction (AMI), accounts for largest causes of death worldwide (Mathers and Loncar, 2006). Reperfusion therapy, including thrombolytic therapy, coronary artery bypass grafting and percutaneous coronary intervention, is believed to be the optimal method on the treatment of AMI (Bassand et al., 2005). However, the prompt reperfusion may lead to further damage of ischemic myocardium, known as myocardial ischemia reperfusion injury (MIRI) (Jennings, 2013). To date, there is still no effective method to prevent MIRI in clinical practice probably in that multiple cardiovascular risk factors, including hypertension, hypercholesterolemia, diabetes and aging, affect the development of MIRI (Ferdinandy et al., 2014).

Previous studies have found that hypercholesterolemia not only contributes to the progression of coronary heart disease, but also may aggravate MIRI (Golino et al., 1987; Tilton et al., 1987). Furthermore, hypercholesterolemia may abrogate the protective effect of preconditioning and postconditioning (Iliodromitis et al., 2006; Kupai et al., 2009; Szilvassy et al., 1995; Ueda et al., 1999). Some reports have demonstrated that hypercholesterolemia exacerbates MIRI involved in increased myocardial oxidative stress and inflammation, attenuation of cell-survival pathways and induction of apoptosis (Liu et al., 2004; Osipov et al., 2009; Wang et al., 2002). However, the potential mechanism that hypercholesterolemia enhances the susceptibility to MIRI is not well understood.

The endoplasmic reticulum (ER) acts important roles in regulation of calcium homeostasis, lipid synthesis, and protein folding (Minamino et al., 2010). ER stress is usually activated in the condition of MIRI and moderate ER stress is protective for cell survival, but severe or prolonged ER stress may lead to apoptosis via ER stress-mediated apoptosis pathway which is a novel apoptosis pathway independent of the death-receptor and mitochondria mediated-apoptosis pathways (Thuerauf et al., 2006; Xu et al., 2005). Recent studies have found that hypercholesterolemia or hypertriglyceridemia aggravates ER-stress response and activates ER stress-mediated apoptosis pathway in macrophages, hepatocytes and pancreatic acinar cells (Feng et al., 2003; Wang et al., 2011; Zeng et al., 2012). Therefore, we infer that hypercholesterolemia may exacerbate MIRI via aggravating ER-stress response and activating the ER stress-related apoptosis pathway. In order to demonstrate this hypothesis, our study firstly detected the effects of hypercholesterolemia on the infarct size, hemodynamic changes, release of myocardial enzyme

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and apoptosis in the condition of MIRI, and then further investigated the roles of ER-stress response and ER stress-mediated apoptosis pathway in this process.

2. Materials and methods

2.1. Animals

All the experimental procedures concerning animals were performed strictly in adherence to the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH). The study procedure was approved by the institutional Ethics Committee of China Medical University (Shenyang, China).

2.2. Induction of experimental hypercholesterolemia

Sixty male Wistar rats weighing 200 ± 10 g were randomly divided into hypercholesterolemia (HC) group and normocholesterolemia (NC) group. Animals in HC group were fed with a diet including 1.5% cholesterol, 5% egg yolk powder, 10% lard, 0.5% sodium cholate, 3% sugar, and 80% normal feedstuff for 8 weeks, and this formula was based on our previous report (Wu et al., 2014), whereas animals in NC group received a normal diet (Qian Ming Experimental animal feed factory, Shenyang, China) for the same period. At the end of 8-week feeding period, blood samples were collected from the rats' vena caudalis for determination of serum levels of total cholesterol (TC), high density lipoprotein (HDL) and low density lipoprotein (LDL) using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

2.3. Heart preparation

Rats were anesthetized through an intraperitoneal injection of 10% chloral hydrate (4 ml/kg). Heparin (1500 IU/kg) was administered intravenously priors to the surgery to prevent intracoronary clot formation. After opening the chest, the heart was rapidly excised and immediately immersed in ice-cold heparinized Krebs–Henseleit solution (KH solution: 127 mmol/l NaCl, 17.7 mmol/l $NaHCO_3$, 5.1 mmol/l KCl, 1.5 mmol/l $CaCl_2$, 1.26 mmol/l $MgCl_2$, 11 mmol/l D-glucose, pH = 7.4) for trimming. Then the heart was mounted on a Langendorff-perfusion apparatus and retrogradely perfused through the aorta with KH solution saturated with 95% O_2–5% CO_2 under a constant pressure of 75 mm Hg at 37 °C. The fluid-filled latex balloon was inserted in the left ventricle via the left atrium for pressure measurement. The balloon was connected to a pressure transducer and the homodynamic parameters including heart rate (HR), left ventricular developed pressure (LVDP), positive first order derivative of ventricular pressure (+dp/dt) and negative first order derivative of ventricular pressure (−dp/dt) were continuously recorded and digitally processed via a homodynamic system (BIOPAC MP150, USA).

2.4. Experimental protocol

Rats in NC and HC groups were further assigned to two subgroups: (i) Control group: the isolated rat hearts were allowed for 20 min of stabilization, and then perfused with KH solution for 150 min without ischemia; (ii) ischemia reperfusion (IR) group: the isolated rat hearts were also allowed for 20 min of stabilization and subjected to 30 min of global ischemia and 120 min of reperfusion.

2.5. Measurement of infarct size

Measurement of infarct size was previously described (Jia, 2011). Briefly, at the end of reperfusion, the heart was harvested and stored at −20 °C for 1 h. The whole heart was sectioned from apex to base into 1–2 mm sections, and incubated in 1% triphenyltetrazolium chloride (TTC) solution for 20 min at 37 °C, fixed by 4% paraformaldehyde for 24 h, and then photographed by a digital camera. The infarct myocardium tissues were unstained and turned into white, whereas viable myocardium tissues were stained and presented red.

Table 1
Serum lipid analysis.

	Total cholesterol (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Normocholesterolemia group	55.4 ± 6.2	22.4 ± 4.6	26.4 ± 5.8
Hypercholesterolemia group	454.2 ± 22.8*	24.6 ± 8.3	395 ± 28.6*

Data were expressed as mean ± SD, n = 20 for each group.
* Indicates $P < 0.05$, hypercholesterolemia group vs. normocholesterolemia group.

2.6. Determination of myocardial enzyme

The release of creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) in coronary effluents collected at the end of reperfusion were determined using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

2.7. Determination of myocardial apoptosis

The specific procedure for determination of myocardial apoptosis was previous described (Ren et al., 2012). Briefly, myocardial apoptosis was detected using In Situ Cell Death Detection Kit (Roche, USA) according to the manufacturer's instructions. The apoptotic nuclei were stained dark brown. In contrast, the normal nuclei were presented blue. Three sections from each myocardial sample were randomly selected and 10 microscopic fields (Olympus BX51 microscope) per section were evaluated by two independent blind observers. For each field, the number of nuclei was counted and the percentage of TUNEL-positive nuclei was calculated.

Table 2
Hemodynamic parameters changes before and during reperfusion.

Time	Baseline	R-30	R-60	R-120
HR				
Control-NC	250 ± 15	235 ± 12	227 ± 15	218 ± 14
Control-HC	252 ± 16	230 ± 15	223 ± 14	212 ± 12
IR-NC	248 ± 18	168 ± 18	172 ± 20	156 ± 18
IR-HC	255 ± 20	172 ± 16	175 ± 18	152 ± 15
LVDP (mm Hg)				
Control-NC	98 ± 8.5	92 ± 6.4	86 ± 7.6	82 ± 8.2
Control-HC	95 ± 7.8	90 ± 6.0	88 ± 7.2	80 ± 6.5
IR-NC	96 ± 8.4	45 ± 5.8	44 ± 4.5	37 ± 5.5
IR-HC	97 ± 9.8	41 ± 6.5	42 ± 6.6	38 ± 4.8
+dp/dt (mm Hg/s)				
Control-NC	2330 ± 230	2265 ± 200	2232 ± 192	2112 ± 178
Control-HC	2310 ± 275	2230 ± 214	2240 ± 185	2090 ± 168
IR-NC	2360 ± 244	1565 ± 195	1626 ± 228	1520 ± 203
IR-HC	2345 ± 262	1610 ± 205	1665 ± 212	1536 ± 228
−dp/dt (mm Hg/s)				
Control-NC	1580 ± 204	1558 ± 165	1510 ± 204	1458 ± 195
Control-HC	1612 ± 185	1575 ± 183	1542 ± 178	1480 ± 192
IR-NC	1586 ± 202	1120 ± 212	1202 ± 185	1165 ± 210
IR-HC	1645 ± 194	1080 ± 185	1164 ± 210	1145 ± 185

IR, ischemia reperfusion; NC, normocholesterolemia; HC, hypercholesterolemia; HR, heart rate; LVDP, left ventricular developed pressure; +dp/dt, positive first order derivative of ventricular pressure; −dp/dt, negative first order derivative of ventricular pressure. Values are means ± SD (n = 8–10).