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Heart protective effects and mechanism of quercetin preconditioning on anti-myocardial ischemia reperfusion (IR) injuries in rats

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ABSTRACT

In this study, we investigated the effects and mechanism of quercetin preconditioning on anti-myocardial ischemia reperfusion (IR) injuries in vivo. Meanwhile, their potential anti-oxidative stress and anti-inflammation effect were assessed. SD rats were orally given quercetin 250 mg/kg. Myocardium apoptosis was determined with TUNEL staining. The biomarkers related to myocardial ischemia injury were determined. Simultaneously, hemodynamic parameters were monitored as left ventricular systolic pressure (LVSP), LV end-diastolic pressure (LVEDP) and maximal rate of increase and decrease of left ventricular pressure (dP/dtmax). The oxidative stress indicators and inflammatory factors were also evaluated. Western blot method was used for analysis of PI3K, Akt, p-Akt, Bax and Bcl-2 protein expressions. The results showed that quercetin significantly reduced apoptosis rate, improved cardiac function, decreased levels of creatine kinase (CK), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH). Quercetin also restrained the oxidative stress related to myocardial ischemia injury as evidenced by decreased malondialdehyde (MDA), and elevated GSH, superoxide dismutase (SOD), catalase (CAT), glutathione-peroxidase (GSH-Px), glutathione reductase (GR) activity. Meanwhile, the inflammatory cascade was inhibited as evidenced by decreased cytokines such as tumor necrosis factor-α (TNF-α), C-reactive protein (CRP) and interleukin-1β (IL-1β). Our results still showed that quercetin pretreatment significantly inhibited the apoptosis by decreasing the number of apoptotic cells, decreasing the level of cleaved Bax, and increasing the level of Bcl-2 in rats subjected to I/R injury. Simultaneously, quercetin pretreatment markedly increased the phosphorylation of Akt. Blockade of PI3K activity by LY294002, dramatically abolished its anti-apoptotic effect and lowered Akt phosphorylation level. It can be concluded that quercetin pretreatment was protected against myocardium IR injury by decreasing oxidative stress, repressing inflammatory cascade, inhibiting apoptosis in vivo and PI3K/Akt pathway involved in the anti-apoptotic effect.

1. Introduction

Ischemic heart disease secondary to acute myocardial infarction is a severe health problem in the world, which is a primary cause of morbidity and mortality (Zweier and Talukder, 2006). In 1960, there was a study firstly to report reperfusion of ischemic myocardium might aggravate myocardial injury in dogs (Jennings et al., 1960). Although timely reperfusion is essential for the salvage of dying myocardium, however, sudden restoration of blood flow to ischemic myocardium may exaggerate myocardial injury paradoxically. This phenomenon is known as myocardial ischemia/reperfusion (I/R) injury (Kambe et al., 2009). It is commonly believed that a number of factors including oxygen radicals, calcium overload, etc. contribute to the pathological process of myocardial I/R injury.

Oxidative stress is usually associated with increased formation of reactive oxygen species (ROS). Oxygen radicals could react with membrane phospholipids, proteins, nucleic acids and other cellular components, acting on the membrane fatty acids, further generate lipid free radicals and lipid peroxides, and damage cell structure and function, leading to cell damage (Roth et al., 1997; Zhao et al., 1996). In ischemia and reperfusion of the heart, oxygen derived free radicals are thought to play an important role in the genesis of tissue injury (Banerjee et al., 2002; Thompson and Zweier, 1990; Visioli et al., 2000; Zweier, 1988). Many reports have demonstrated that free radical scavengers reduced free radical injury in the ischemic–reperfused heart (Chambers et al., 1989; Gelvan et al., 1991; Janero and Burghardt, 1989; Packer et al., 1991; Pyke and Chan, 1990; Rezanick et al., 1992), which supports the potential therapeutic uses of the free radical scavengers in this condition.

The cardiomyocyte apoptosis and inflammatory reaction have been recognized as hallmarks of myocardial reperfusion injury. Recent evidences suggest that myocardial apoptosis is initiated shortly after

Abbreviations: IR, ischemia reperfusion; LVSP, left ventricular systolic pressure; LVEDP, LV end-diastolic pressure; dP/dtmax, maximal rate of increase and decrease of left ventricular pressure; CK, creatine kinase; LDH, lactate dehydrogenase; MDA, malondialdehyde; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α; CRP, C-reactive protein; IL-1β, interleukin-1β; NO, nitric oxide; LAD, left anterior descending coronary artery.

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ischemia, is amplified by reperfusion, and partially contributes to overall cardiomyocyte death (Fliss and Gattinger, 1996). Blocking the apoptotic process may prevent the loss of contractile cells, minimize cardiac injury induced by I/R, and slow the occurrence of myocardial stunning and heart failure (Anselmi et al., 2004). Likewise, reducing inflammatory responses during reperfusion after ischemic insult has been shown to be beneficial in numerous studies (Bao et al., 2004; Sun et al., 2012).

Activation of the PI3K/Akt pathway has been reported to prevent neuronal apoptosis and protect the brain from cerebral ischemia/reperfusion (I/R) injury (Hua et al., 2008; Lu et al., 2011). Phosphoinositide-3-kinase (PI3K)-Akt signaling pathway plays a crucial role in cell growth and cell survival. The PI3K-Akt signaling pathway can be activated by many types of cellular stimuli or toxic insults (Porta and Figlin, 2009). Serine/threonine kinase Akt/PKB is the primary mediator of PI3K-initiated signaling. Activated Akt by PI3K regulates cell survival through phosphorylation of a variety of downstream targets such as pro-apoptotic protein, transcription factors and another protein kinase (Franke et al., 2003; Ou et al., 2010). Akt can activate endothelial nitric oxide synthase (eNOS), which leads to nitric oxide (NO) production (Fulton et al., 1999; Ou et al., 2010). The PI3K/Akt pathway can also mediate some of its survival signals through the Bcl-2 family (Limaye et al., 2005).

Quercetin (3,5,7,3′,4′-pentahydroxyflavone) is one of the major flavonoids found in many vegetables and fruits such as onion and apple. It has various biological functions including anti-oxidative (Robak and Gryglewski, 1988), anti-inflammatory, anti-coagulation, and oxygen radical-scavenging activities (Erden Inal and Kahraman, 2000). Animal studies demonstrated that quercetin exerts vasodilating and blood pressure-lowering effects in spontaneous hypertensive rats (Duarte et al., 2001; Sanchez et al., 2006) and in rats fed a high-fat high sucrose diet (Yamamoto and Oue, 2006). Rat models of MI/R were induced by coronary occlusion followed by reperfusion, treatment of rats with quercetin (1.0 mg/kg, i.v.) induced a significant reduction of infarct volume and improvements in baseline hemodynamic abnormalities ($P < 0.05$). Quercetin treatment also attenuated the expression of both TNF-alpha (TNF-α) and interleukin-10 (IL-10) and lowered the serum levels of inflammatory cytokine ($P < 0.05$) (Jin et al., 2012). Annapurna et al. (2009) report that quercetin and rutin significantly limit the myocardial infarct size in both normal and diabetic animals in a similar fashion. Wan et al. (2009) report that quercetin not only inhibited myocardial ischemia–reperfusion-induced NOX2 and iNOS mRNA and protein expression but also inhibited eNOS mRNA and protein expression. Ikizler et al. (2007) report that quercetin has the capacity to protect the myocardial tissue against global ischemia and reperfusion injury.

So far, no report is available for its preconditioning effect on myocardial I/R injury and PI3K/Akt signals in vivo. In the current study, therefore, we characterized the cardioprotective properties of quercetin and provided evidences that these cardioprotective effects were in part mediated through PI3K/Akt signaling pathways.

2. Material and methods

2.1. Material

Quercetin was purchased from Xian Huipu Plant Ltd, Xian City, China. Its purity is 93%.

2.2. Experimental protocol for drug pretreatment

Rats were randomly assigned to undergo sham surgery (sham group) or ischemia–reperfusion. For those undergoing ischemia–reperfusion, some rats (10 rats in each group) were given saline by oral gavage (IR control group) for 10 days before IR operation; some rats were administered with quercetin (250 mg/kg) + LY294002 (an inhibitor of PI3K, 0.2 mg/kg, s.c.) or LY294002 (an inhibitor of PI3K, 0.2 mg/kg, s.c.) by oral gavage for 10 days before IR operation.

The animals were anesthetized with pentobarbital (35 mg/kg, i.p.) and, after tracheotomy, ventilation was provided using a breathing machine at a respiratory rate of 50/min with a tidal volume of 15 mL/kg body weight. Blood pressure was recorded from the left common carotid artery using a pressure transducer, and the heart rate was monitored by an electrocardiogram (ECG) during the procedure. A left parasternal incision was made through the third and fourth ribs, and the pericardium was gently opened to expose the heart. The left anterior descending coronary artery (LAD) was ligated using a 6–0 silk suture. Additionally, a medical latex tube (socket, inner diameter, 1.5 mm) was placed between the ligature and LAD. Myocardial ischemia was induced by compressing the LAD by tightening ligature around latex tube. The ECG was monitored for changes in the ST-T segment caused by tightening or loosing the ligature. After 30 min ischemia, the latex tube was removed in order to restore the coronary circulation. At 4 h post-reperfusion, rats were sacrificed, and parts of the ischemic anterior wall of left ventricular myocardium near cardiac apex and blood samples were obtained for further analysis. The sham-operated group underwent the same procedures, except the silk suture was left untied.

2.3. Measurement of antioxidant indices

SOD, CAT, GSH-Px, GR activities and GSH, MDA contents were used as indices of reactive oxygen species and membrane lipid peroxidation level. The content of MDA, GSH and activities of SOD, CAT, GSH-Px, GR were measured using commercial kits (JianCheng Bioengineering Institute, Nanjing, China) and analyzing with a spectrophotometer. Detailed manipulation process was performed according to the manufacturer's instructions.

2.4. Determination of release of AST, LDH, CK-MB into serum

Myocardial cellular damage was evaluated by measuring serum AST, LDH, and CK-MB levels. Serum AST and LDH activities were measured spectrophotometrically, and serum CK-MB was quantified using a commercial ELISA kit according to the manufacturer's instructions.

2.5. Determination of serum TNF-α, CRP, IL-1β

The levels of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), C-reactive protein (CRP), and interleukin-1β (IL-1β) in the serum samples were quantified using specific ELISA kits for rat according to the manufacturers' instructions. Serum levels of TNF-α and IL-1β were calculated from the kit standards and expressed in pg/mL, while CRP was expressed in μg/mL.

2.6. Determination of Ca^{2+}–ATPase and Na^{+}–K^{+}–ATPase activity

Activities of Na^{+}–K^{+}–ATPase and Ca^{2+}–ATPase from cardiac tissues were determined by the method of Bonting (1970) and Hjerten and Pan (1983), respectively. The activities were indirectly measured by estimating the phosphorous liberated after the incubation of cardiac tissue homogenate in a reaction mixture containing the substrate ATP with the co-substrate elements at 37 °C for 15 min. The reactions were arrested by adding 1.0 mL of 10% trichloroacetic acid (TCA). The phosphorus content from the TCA supernatants was then determined by the method of Fiske and Subbarow (1925).

2.7. Determination of myocardial apoptosis

A terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was performed using an In Situ Cell Death Detection kit according to the manufacturer's instructions. Both positive (DNase-treated) and negative (no addition of terminal transferase) control tissue sections