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

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A B S T R A C T

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.

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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...
ischemia, is amplified by reperfusion, and partially contributes to overall cardiomyocyte death (Fliss and Gattlinger, 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 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, 2000). Serine/threonine kinase Akt/PKB is the primary mediator of PI3K-initiated signaling. Activated PI3K by Akt 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 (Lumeye 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 (Enral Inal and Kahrman, 2000). Animal studies demonstrated that quercetin exerts vasodilating and blood flow-stimulating activities (Yamamoto and Oue, 2006). Rat models of MI/R were induced by coronary artery ligation (Yamamoto and Oue, 2006). Rat models of MI/R were induced by coronary artery 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-α (TNF-α) and interleukin-10 (IL-10) and lowered the serum levels of inflammatory cytokine (P < 0.05) (Jin et al., 2012). Annaupurana 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 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 loosening 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 Ca2⁺–ATPase and Na⁺–K⁺–ATPase activity

Activities of Na⁺–K⁺–ATPase and Ca²⁺–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 phosphoryl 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
Table 1
Effect of quercetin pre-treatment on LVSP, LVEDP, +dp/dtmax and dp/dtmax.

<table>
<thead>
<tr>
<th>Group</th>
<th>LVSP (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>+dp/dtmax (mm Hg/s)</th>
<th>−dp/dtmax (mm Hg/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>127 ± 11</td>
<td>7.04 ± 0.66</td>
<td>4517.39 ± 402.31</td>
<td>−4128.36 ± 426.15</td>
</tr>
<tr>
<td>IR</td>
<td>99.24 ± 8.48</td>
<td>16.33 ± 1.52</td>
<td>3061.58 ± 327.14</td>
<td>−3126.38 ± 309.62</td>
</tr>
<tr>
<td>IR + LY29400</td>
<td>90.62 ± 7.91</td>
<td>18.36 ± 1.49</td>
<td>2895.17 ± 227.08b</td>
<td>−2875.94 ± 269.72b</td>
</tr>
<tr>
<td>IR + quercetin</td>
<td>119.83 ± 10.05</td>
<td>10.91 ± 0.11c</td>
<td>3884.02 ± 351.52c</td>
<td>−3739.14 ± 300.84c</td>
</tr>
<tr>
<td>IR + quercetin + LY29400</td>
<td>104.71 ± 11.47</td>
<td>14.61 ± 1.74</td>
<td>3296.71 ± 333.63b</td>
<td>3253.47 ± 342.17b</td>
</tr>
</tbody>
</table>

* p < 0.01, compared with sham group.
+ p < 0.05.
* p < 0.01, compared with IR group.
+ p < 0.05.
* p < 0.01, compared with IR + quercetin group.

Table 2
Effect of quercetin pre-treatment on myocardium apoptosis rate.

<table>
<thead>
<tr>
<th>Group</th>
<th>Apoptosis index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>1.82 ± 0.15</td>
</tr>
<tr>
<td>IR</td>
<td>21.59 ± 1.89a</td>
</tr>
<tr>
<td>IR + LY29400</td>
<td>33.16 ± 2.73a</td>
</tr>
<tr>
<td>IR + quercetin</td>
<td>12.35 ± 1.02b</td>
</tr>
<tr>
<td>IR + quercetin + LY29400</td>
<td>18.37 ± 1.63c</td>
</tr>
</tbody>
</table>

* p < 0.01, compared with sham group.
+ p < 0.01, compared with IR group.
* p < 0.05.
+ p < 0.01, compared with IR + quercetin group.

Table 3
Effect of quercetin pre-treatment on myocardium apoptosis rate.

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (nmol/mg)</th>
<th>GSH (μg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>9.26 ± 0.63</td>
<td>100.26 ± 8.93</td>
</tr>
<tr>
<td>IR</td>
<td>25.28 ± 2.13a</td>
<td>46.83 ± 2.84b</td>
</tr>
<tr>
<td>IR + LY29400</td>
<td>31.02 ± 2.89b</td>
<td>38.35 ± 2.77b</td>
</tr>
<tr>
<td>IR + quercetin</td>
<td>13.39 ± 1.16b</td>
<td>89.29 ± 6.93b</td>
</tr>
<tr>
<td>IR + quercetin + LY29400</td>
<td>21.14 ± 1.80c</td>
<td>66.93 ± 5.82b</td>
</tr>
</tbody>
</table>

* p < 0.01, compared with sham group.
+ p < 0.01, compared with IR group.
* p < 0.05.
+ p < 0.01, compared with IR + quercetin group.

3. Results

We demonstrated in the present study that LVSP, +dp/dtmax and dp/dtmax in IR group were significantly lower, whereas LVEDP was significantly higher than those in sham group (Table 1, p < 0.01). Pre-treatment with quercetin significantly (p < 0.01) increased LVSP, +dp/dtmax and dp/dtmax reduced the LVEDP in IR + quercetin group compared to IR group. However, increased LVSP, +dp/dtmax and dp/dtmax, and decreased LVEDP in IR + quercetin + LY29400 group were significantly (p < 0.05, p < 0.01) reversed compared to IR + quercetin group.

We demonstrated in the present study that myocardium apoptosis rate in IR group was significantly higher than that in sham group (Table 2, p < 0.01). Pre-treatment with quercetin significantly (p < 0.01) decreased myocardium apoptosis rate in IR + quercetin group compared to IR group. However, decreased myocardium apoptosis rate in IR + quercetin + LY29400 was significantly (p < 0.01) reversed compared to IR + quercetin group.

In the present study, myocardium MDA level in IR group was significantly higher, whereas GSH level was significantly lower than those in sham group (Table 3, p < 0.01). Pre-treatment with quercetin significantly (p < 0.01) decreased MDA level, increased the GSH level in IR + quercetin group compared to IR group. However, decreased MDA, and increased GSH levels in IR + quercetin + LY29400 group were significantly (p < 0.01) reversed compared to IR + quercetin group.

Results presented in Table 4 indicated that activities of myocardium antioxidant enzymes (SOD, CAT, GSH-Px and GR) in IR group were significantly (p < 0.01) lower than those in sham group. Activities of myocardium antioxidant enzymes (SOD, CAT, GSH-Px and GR) were significantly (p < 0.01) increased in quercetin treated rats compared with IR group rats. However, activities of myocardium antioxidant enzymes (SOD, CAT, GSH-Px and GR) were significantly (p < 0.05, p < 0.01) decreased in rats treated with quercetin and LY29400 compared to quercetin treated rats.

Results presented in Table 5 indicated that levels of serum enzymes, namely AST, CK-MB and LDH, in IR group were significantly (p < 0.01) higher than those in sham group. Levels of serum enzymes (AST, CAT, GSH-Px and GR) were significantly (p < 0.01) decreased in quercetin treated rats compared with IR group rats.
CK-MB and LDH) were significantly (p < 0.01) decreased in quercetin treated rats compared with IR group rats. However, levels of serum enzymes (AST, CK-MB and LDH) were significantly (p < 0.01) increased in rats treated with quercetin and LY29400 compared to quercetin treated rats.

Results presented in Table 6 indicated that levels of serum CRP, TNF-α and IL-1β in IR group were significantly (p < 0.01) higher than those in sham group. Levels of serum CRP, TNF-α and IL-1β were significantly (p < 0.01) decreased in quercetin treated rats compared with IR group rats. However, levels of serum CRP, TNF-α and IL-1β were significantly (p < 0.01) increased in rats treated with quercetin and LY29400 compared to quercetin treated rats.

Results presented in Table 7 indicated that activities of myocardium Na⁺−K⁺−ATPase and Ca²⁺−ATPase in IR group were significantly (p < 0.01) lower than those in sham group. Activities of myocardium Na⁺−K⁺−ATPase and Ca²⁺−ATPase were significantly (p < 0.01) increased in quercetin treated rats compared with IR group rats. However, activities of myocardium Na⁺−K⁺−ATPase and Ca²⁺−ATPase were significantly (p < 0.05) decreased in rats treated with quercetin and LY29400 compared to quercetin treated rats.

The expression of PI3K, Akt and p-Akt proteins in the myocardium tissues are summarized in Fig. 1. Results showed that the IR control group significantly (p < 0.01) decreased the expression level of p-Akt proteins compared with the sham group. The administration of quercetin successfully (p < 0.01) increased the p-Akt protein expression level. The IR + quercetin + LY29400 group registered a significantly lower p-Akt protein expression level (p < 0.01) than the IR + quercetin group. For the PI3K and Akt proteins level, there was no significant difference among the different groups tested.

In order to learn whether quercetin pre-treatment can inhibit myocardial cell death, the expression of proapoptotic Bax and antiapoptotic Bcl-2 protein in myocardium tissue was measured. As shown in Fig. 2, we observed an increase of Bax protein expression, as well as a decrease of Bcl-2 protein expression in IR group in comparison to the sham group (p < 0.01). We also observed a decrease of Bax protein expression upon quercetin pre-treatment (p < 0.05) in comparison to IR control, as well as an increase of Bcl-2 in

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### Table 4

Effect of quercetin pre-treatment on myocardium antioxidant enzyme activities.

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (U/L)</th>
<th>CAT (U/L)</th>
<th>GSH-Px (U/L)</th>
<th>GR (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>261.7 ± 24.6</td>
<td>49.25 ± 4.81</td>
<td>66.28 ± 7.32</td>
<td>52.81 ± 4.44</td>
</tr>
<tr>
<td>IR</td>
<td>126.03 ± 11.7a</td>
<td>18.37 ± 1.93a</td>
<td>27.42 ± 1.96a</td>
<td>30.63 ± 3.41a</td>
</tr>
<tr>
<td>IR + LY29400</td>
<td>162.17 ± 18.3</td>
<td>22.81 ± 2.65</td>
<td>36.07 ± 2.99</td>
<td>37.25 ± 2.97</td>
</tr>
<tr>
<td>IR + quercetin</td>
<td>255.93 ± 29.8b</td>
<td>44.52 ± 4.81b</td>
<td>69.36 ± 4.81b</td>
<td>49.42 ± 4.37b</td>
</tr>
<tr>
<td>IR + quercetin + LY29400</td>
<td>231.04 ± 29.1bc</td>
<td>34.63 ± 4.14ad</td>
<td>47.13 ± 4.28ad</td>
<td>40.11 ± 3.83ad</td>
</tr>
</tbody>
</table>

*p < 0.01, compared with sham group.

### Table 5

Effect of quercetin pre-treatment on serum AST, CK-MB and LDH levels.

<table>
<thead>
<tr>
<th>Group</th>
<th>AST (U/L)</th>
<th>CK-MB (U/L)</th>
<th>LDH (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>439.51 ± 40.52</td>
<td>4908.42 ± 438.07</td>
<td>12747.73 ± 119.69</td>
</tr>
<tr>
<td>IR</td>
<td>793.62 ± 82.63a</td>
<td>13169.65 ± 148.32a</td>
<td>2893.61 ± 306.85a</td>
</tr>
<tr>
<td>IR + LY29400</td>
<td>905.44 ± 78.74ab</td>
<td>16942.04 ± 205.52b</td>
<td>3651.09 ± 336.14b</td>
</tr>
<tr>
<td>IR + quercetin</td>
<td>488.69 ± 47.58a</td>
<td>7062.53 ± 715.57ab</td>
<td>16042.7 ± 152.4b</td>
</tr>
<tr>
<td>IR + quercetin + LY29400</td>
<td>672.82 ± 62.07bc</td>
<td>9041.51 ± 894.36bc</td>
<td>2518.53 ± 261.13bc</td>
</tr>
</tbody>
</table>

*p < 0.01, compared with sham group.

### Table 6

Effect of quercetin pre-treatment on serum CRP, TNF-α and IL-1β levels.

<table>
<thead>
<tr>
<th>Group</th>
<th>CRP (pg/mL)</th>
<th>TNF-α (pg/mL)</th>
<th>IL-1β (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>5.04 ± 0.47</td>
<td>6.94 ± 0.83</td>
<td>511.62 ± 47.51</td>
</tr>
<tr>
<td>IR</td>
<td>13.46 ± 1.54a</td>
<td>23.18 ± 3.11a</td>
<td>804.28 ± 77.57a</td>
</tr>
<tr>
<td>IR + LY29400</td>
<td>126.25 ± 1.92a</td>
<td>31.38 ± 3.63a</td>
<td>915.38 ± 80.73a</td>
</tr>
<tr>
<td>IR + quercetin</td>
<td>6.91 ± 0.53a</td>
<td>10.27 ± 1.33a</td>
<td>632.49 ± 71.05b</td>
</tr>
<tr>
<td>IR + quercetin + LY29400</td>
<td>9.53 ± 0.85bc</td>
<td>17.03 ± 2.28bc</td>
<td>728.29 ± 83.04ad</td>
</tr>
</tbody>
</table>

*p < 0.01, compared with sham group.

### Table 7

Effect of quercetin pre-treatment on myocardium Na⁺−K⁺−ATPase and Ca²⁺−ATPase activities.

<table>
<thead>
<tr>
<th>Group</th>
<th>Na⁺−K⁺−ATPase (μmol-mg protein−1·h−1)</th>
<th>Ca²⁺−ATPase (μmol-mg protein−1·h−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sham</td>
<td>1.55 ± 0.16</td>
<td>1.22 ± 0.13</td>
</tr>
<tr>
<td>IR</td>
<td>0.59 ± 0.06</td>
<td>0.62 ± 0.07</td>
</tr>
<tr>
<td>IR + LY29400</td>
<td>0.33 ± 0.04</td>
<td>0.48 ± 0.05</td>
</tr>
<tr>
<td>IR + quercetin</td>
<td>1.43 ± 0.12</td>
<td>1.04 ± 0.14</td>
</tr>
<tr>
<td>IR + quercetin + LY29400</td>
<td>1.11 ± 0.1bc</td>
<td>0.83 ± 0.09sc</td>
</tr>
</tbody>
</table>

*p < 0.01, compared with sham group.

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comparison to the IR group (p < 0.01). LY29400 treatment significantly increased myocardium Bax protein expression, decreased Bcl-2 protein expression in IR + quercetin group in comparison to IR + quercetin group. In addition, we have calculated the relative ratios of Bax to Bcl-2 (Fig. 3). We found that Bax/Bcl-2 ratio in IR group was significantly higher than that in sham group. Bax/Bcl-2 ratio in IR + quercetin group was markedly lower than that in IR group. However, Bax/Bcl-2 ratio in IR + quercetin + LY29400 group was markedly lower than that in IR + quercetin group.

4. Discussion

The mechanism involved in the cardioprotective action of quercetin is still open to discussion. The present experiments demonstrate that the quercetin pre-treatment alleviates the deterioration of cardiac contractile function, decreases myocardium apoptosis and prevents mortality when given orally for 10 consecutive days to myocardium IR rats.

There is a dynamic relationship between reactive oxygen species (ROS) and antioxidants in the human body (Harrison et al., 2003). Healthy cells can scavenge free radicals effectively by means of antioxidants. However, in pathological conditions, like ischemic–reperfusion injury (IRI), the sudden generation of ROS can dramatically upset this balance with an increased demand on the antioxidant defense system. Once generated, free radicals alter the structural and functional integrity of cells by a variety of mechanisms, including lipid peroxidation, proteolysis and shearing of the nuclear material (Sun et al., 2002). Endogenous antioxidants including, SOD, CAT and GPx are depleted accompanied by accumulation of ROS. It may be possible to limit oxidative stress induced tissue damage and, hence, prevent or ameliorate disease progression by favoring the balance towards lower oxidative stress. In the present study, ischemic–reperfusion injury was associated with increased oxidative stress, as evidenced by increase in myocardial TBARS and depletion of myocardial endogenous antioxidants, like SOD, GSH-Px, GR and CAT. On the other hand, the ROS produced by the IR can be scavenged directly by the quercetin thereby preventing lipid peroxidation and helping to maintain membrane integrity. This is further supported by the decreased levels of CK-MB, AST and LDH in the quercetin treated group.
Among potential inflammatory triggers in acute myocardial ischemia, primary cytokines such as IL-1β and TNF-α induce “messeger” cytokines releasing into circulation to cause the increase of CRP and other acute reactants (Willerson and Ridker, 2004). The elevated pro-inflammatory cytokines like TNF-α and IL-1β during acute myocardial ischemia contribute to depressing LV function and cardiomyocyte loss by apoptosis (Rutschow et al., 2006). The production of CRP following myocardial necrosis is the typical acute phase response to cell death and inflammation (Hirschfield and Pepys, 2003). In our study, inflammatory factors such as CRP, IL-1β and TNF-α were secreted increasingly in the serum of control group, but this inflammatory cascade was markedly repressed by quercetin. It indicates that quercetin pre-treatment decreases serum inflammatory cytokines in rat after myocardial ischemia injury.

The abnormalities in Na⁺/K⁺-ATPase and Ca²⁺-ATPase activities are well documented in cardiac dysfunction (Sapia et al., 2010; Wu et al., 2010). The Ca²⁺-ATPase is the major active calcium transport protein responsible for the maintenance of normal intracellular calcium levels in a variety of cell types. The Na⁺/K⁺-ATPase (sodium pump) is a heterodimer protein that plays a key role in the maintenance of cell homeostasis by regulation of membrane potential and cation transport across the sarcolemmal membrane. Depressed Na⁺/K⁺-ATPase activity is observed after treatment of cardiac membranes with oxidants such as hypochlorous acid (Kato et al., 1998; Kukreja et al., 1990) and H₂O₂ (Matsouka et al., 1990; Oguro et al., 1992; Shao et al., 1995) as well as xanthine plus xanthine oxidase (XXO), which is known to generate oxyradicals (Shao et al., 1995; Xie et al., 1990).

In the present study, the observed decrease in the activity of Na⁺/K⁺-ATPase and Ca²⁺-ATPase may be considered as an index of cardiovascular damage induced by IR. Our studies have found that supplementation with quercetin prevents lipid peroxidation, protein glycation and inhibition of Na⁺/K⁺-ATPase and/or Ca²⁺-ATPase activity caused by myocardial IR, indicating that quercetin increases cardiac pump function.

Recent studies have shown that activation of the PI3K/Akt signaling pathway protects the myocardium from myocardial IR injury and prevents cardiac myocyte apoptosis (Cao et al., 2013; Xu et al., 2009). Activation of the PI3K/Akt signaling pathway can suppress the lipopoly-saccharide (LPS)-induced inflammatory response and activation of coagulation in endotoxemic mice (Liu et al., 2010). We observed in the present study that administration of quercetin significantly increased the levels of p-Akt in the myocardium, suggesting that quercetin activates the PI3K/Akt signaling pathway. Of note, PI3K has been confirmed to be involved in growth factor signal transduction via receptor and non-receptor tyrosine kinases. Hence, inhibitors of PI3K may potentially give a better understanding of the function and regulatory mechanisms of PI3K. The compound, LY294002, has been proven to specifically abolish PI3K activity with a selective structure activity relationship and with slight changes in structure (Xu et al., 2009). Blocking the PI3K activity by LY294002 abolished the protective effect of quercetin. Collectively, these data suggest that quercetin-induced prevention of cardiac dysfunction is mediated in part via a PI3K/Akt dependent mechanism.

The balance of the expression of anti- and pro-apoptotic members of the Bcl-2 gene family dictates the susceptibility of the cells to a variety of apoptotic stimuli (Almeida et al., 2000). IR causes myocardial death including apoptosis of cardiomyocytes (Morkuniene et al., 2006). Current studies indicate that IR promotes apoptosis of cultured cardiomyocytes accompanied by a down-regulation of Bcl-2 (protein) with a simultaneous increase of Bax (protein). The findings suggest that IR may increase myocyte apoptosis via decrease of the ratio of Bcl-2/Bax. Based on finding of an anti-apoptotic effect of quercetin on IR-induced apoptosis (Stanely Mainzen Prince and Sathya, 2012; Zaafan et al., 2013), here we showed that quercetin may increase myocyte apoptosis via both inhibition of synthesis of Bcl-2 and promotion of production of Bax in the cardiomyocytes. Quercetin attenuates the IR induced apoptosis mainly through restoration of the synthesis of Bcl-2 and the ratio of Bcl-2/Bax back to its normal level.

In conclusion, this study promotes the understanding of the myocardium-protective effect of quercetin in IR rats. The findings of current study demonstrate that quercetin improves heart function, decreases myocardial oxidative injury, apoptosis of cardiomyocytes in concert with increase of Bax and inhibition of Bcl-2, leading to a reduction of the ratio of Bcl-2/Bax in the myocytes. We suppose that quercetin plays its myocardium-protective effect by increasing phospho-Akt, Bcl-2 levels and decreasing Bax levels and the ratio of Bcl-2/Bax in heart.

Conflict of interest
None.

References


Shao, Q., Matsubara, T., Bhatt, S.K., Dhalli, N.S., 1995. Inhibition of cardiac sarcoplasmic Na+-K+-ATPase by oxyradical generating systems. Molecular and Cellular Biochemistry 147, 139–144.


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