The protective effect of peony extract on acute myocardial infarction in rats

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A B S T R A C T
To investigate the protective effects, and the mechanisms involved, of an extract of the medicinal herb Radix paeoniae rubra (PE) on cardiovascular disease, acute myocardial infarction (AMI) was induced by ligation of the left coronary artery in Sprague Dawley rats. Animals were randomly divided into six groups: control, sham-operated, AMI, AMI + PE low dose, AMI + PE high dose, and AMI + positive control. Cardiomyocytes were examined. In contrast with control and sham groups, significant increases in the following parameters were measured in the blood of AMI group animals: activities of cardiac enzymes including glutamic oxaloacetic transaminase, creatine kinase, creatine kinase-MB, lactate dehydrogenase, α-hydroxybutyric dehydrogenase, and levels of IL-10, TNFα, and lipid peroxidation. Under the same conditions, superoxide dismutase activity, thrombin time and activated partial thromboplastin time decreased significantly.

Introduction

Radix paeoniae rubrae is a medicinal herb that has been used in China and other Asian countries for thousands of years for treating various diseases, including obesity and diabetes (Jiang et al., 2009; Zhang et al., 2009; Zheng et al., 2008), hepatitis, arthritis (Zheng and Wei, 2005), dementia, and traumatic injuries (Liu et al., 2005). This herb has been suggested to possess anti-allergic, anti-inflammatory, anti-oxidant, and immunoregulatory effects (Jiang et al., 2009; Su et al., 2010; Zheng et al., 2008). The effects have been attributed to the total glucosides in peony, a group of monoterpenoid glycosides. Paeoniflorin (PF) has been considered a major component of these total glucosides. In a previous study, we found that peony extract showed protective effects against isoprenaline-induced myocardial ischemia in rats (Shen et al., 2007), suggesting it may be beneficial for treating cardiovascular diseases.

Cardiovascular disease is highly prevalent in the elderly. Acute myocardial infarction (AMI), a kind of coronary heart disease, can be induced by coronary artery occlusion in animals (Yang et al., 2010). In the present study, we induced AMI by permanent ligation of the left coronary artery in rats and studied both the protective effects of peony extract (PE) and the possible mechanisms involved.

Methods

Chemicals and reagents

Isosorbide dinitrate was purchased from Nanjing Baijingyu Ltd., Inc. (Nanjing, China); Annexin V-FITC from Beijing Aobo Biotechnology Ltd., Inc. (Beijing, China); kits for Bradford protein determination and caspase-3 activity from Beyotime Biotechnology Ltd., Inc. (Nanjing, China); total RNA extract and RNA Fixer from Beijing Biotech Ltd., Inc. (Beijing, China); cDNA first strand synthesizer from Fermentas (Glen Burnie, MD, USA); Taq DNA Polymerase from Guangdong Dongsheng Ltd., Inc. (Guangzhou, China); Annexin V-FITC from Beijing Aobo Biotechnology Ltd., Inc. (Beijing, China); ELISA kits for assaying IL-10 and TNFα from Shanghai Senxiong Technology Industry Ltd., Inc. (Shanghai, China); malondialdehyde (MDA) and superoxide dismutase (SOD) assay kits from Nanjing Jiancheng Biotechnology (Nanjing, China); glutamic oxaloacetic transaminase (GOT), creatine kinase isoenzyme MB (CK-MB), creatine kinase (CK), lactate dehydrogenase (LDH) and alpha-hydroxybutyric dehydrogenase (α-HBDH) assay kits.
from Beijing Zhongsheng Beikong Biotechnology Ltd., Inc. (Beijing, China); Thrombin Time (TT), Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) assay kits from Shanghai Sun Biotechnology Ltd., Inc. (Shanghai, China). Other chemicals and reagents were obtained from local vendors in the highest available quality.

**PE preparation**

PE was prepared from the dried root of peony plant with 70% ethanol as previously described (Piao et al., 2007). The PE qualitative and quantitative analyses of the paeoniflorin, the major component of PE, were performed by RP-HPLC with UV detection as described by Wu et al. (2009) and a UV spectrometer. The stock solution contained 25 mg/ml of paeoniflorin in water.

**Experimental animals**

Male and female Sprague Dawley rats (40 of each gender) weighing 170–210 g were purchased from the Experimental Animal Center at Xi’an Jiaotong University Medical School. The 80 rats were randomly divided into 6 groups: normal control (Control), sham operation (Sham), injured (AMI), PE low dose (AMI + PE-L), PE high dose (AMI + PE-H) and isosorbide dinitrate (AMI + isosorbide). The acute myocardial infarction animal model was established as same as AMI but not ligated. The animals except the normal control group received distilled water or drugs by intragastric administration for 6 days (2 ml/time, twice/day). Doses for AMI + PE-L, AMI + PE-H and AMI + isosorbide groups were 5 mg/ml, 25 mg/ml and 25 mg/ml, respectively.

**Determination of cardiac function**

Cardiac function was determined with the methods described previously (Wang et al., 2010). Powerlab data analysis system (Australia AD-instruments) was utilized to record blood pressure (BP), left ventricular end-diastolic pressure (LVEDP), left ventricular systolic pressure (LVSP) and maximum change rate of left ventricular pressure rise and fall (±dp/dtmax) by choosing at least 5 continuous waves at the stationary phase.

**Serum preparation**

Blood was obtained from the abdominal aorta of rats after anaesthetization. A portion of blood was centrifuged at 4 °C and 3000 rpm for 10 min after sitting at room temperature for 30 min. The supernatant was taken out and stored at −20 °C prior to assay of CK-MB, CK, LDH, α-HBDH, GOT, MDA, SOD, TNF-α and IL-10. Another portion of blood was gently mixed with natrium citricum (0.109 mol/l) in a polyethylene tube and centrifuged at 3000 rpm for 15 min. Supernatants were taken out and stored at −80 °C prior to measurement of TT, PT and APTT.

**Serum biochemical measurements of cardiac enzymes, cytokines, and oxidative stress**

The activities of cardiac enzymes including GOT, CK-MB, CK, LDH and α-HBDH were determined spectrophotometrically using diagnostic kits in accordance with the manufacturer’s instructions. Levels of TNF-α and IL-10 were determined with ABC-ELISA kits. SOD activity was measured with a xanthine oxidase system and MDA levels were measured with the thiobarbiturilum assay. TT, PT and APTT were carried out using commercial kits.

**Preparation of primary cardiomyocytes from heart muscle**

Cardiomyocytes were prepared from heart after measurement of cardiac functions as described previously (Shen et al., 2007).

**Detection of early stage apoptosis in cardiomyocytes**

Early stage apoptosis was detected with the FITC-Annexin-V/PI method (Shen et al., 2007). The degree of early stage apoptosis was calculated from the ratio of the number of apoptotic to the number of non-apoptotic cardiomyocytes.

**Measurement of caspase-3 activity in cardiomyocytes**

The activity of caspase-3 was measured following the kit manufacturer’s instructions and normalized with the protein concentration.

**Measurement of expression levels of the apoptosis-related genes bcl-2, bax and fas in cardiomyocytes**

Total RNA of myocardium was extracted with Trizol. First strand cDNA was synthesized according to a protocol. A reaction system (20 µl) containing synthetic oligonucleotide primers was used to amplify the desired genes (bcl-2, bax and fas). Following agarose gel electrophoresis, the bands were analyzed with Bandscan 5.0.

**Results**

**RP-HPLC and UV spectrometric analytical results of PE**

The major components of PE are total peony glycosides. It is well known that paeoniflorin is the most abundant glycoside in the extract and is used as the index for PE quality control. The other minor components with much lower levels than paeoniflorin include oxypaeoniflorin, benzoylpaeoniflorin, oxybenzoyl-paeoniflorin, alibiflorin, paeoniflorigenone, and lactiflorin (Su et al., 2010). We examined our PE with RP-HPLC and UV detection following the method described previously (Wu et al., 2009). As shown in Fig. 1, the retention time of the pure paeoniflorin appeared at 4.437 min (Fig. 1A) and that of the major peak of the PE appeared at 4.378 min (Fig. 1B). The difference was within the range of instrumental error. UV spectrometric analysis showed that PE sample and pure paeoniflorin had similar UV spectra with absorption peak at 230–231 nm. Quantitative analysis found that the concentration of paeoniflorin in the Radix paeoniae rubra is 3.4%, which is consistent with previous reports (Wu et al., 2009; Su et al., 2010) and also with the quality requirement of national pharmacopedia of China.

**The effect of PE on serum cytokines**

Levels of both TNF-α and IL-10 in the AMI group were significantly increased over those in normal control or sham-operated animals (p < 0.001). Treatment with PE low dose and PE high dose, just as with isosorbide dinitrate, both inhibited the AMI-induced increases in serum TNF-α (Fig. 2A) and IL-10 (Fig. 2B). TNF-α was lowered by PE low dose, PE high dose and isosorbide dinitrate to 62.5%, 60.2% and 77.1% of the AMI group level, respectively; similarly, IL-10 was lowered by PE low dose, PE high dose and isosorbide dinitrate to 85.2%, 70.7% and 87.6% of the AMI group level, respectively.

**The effect of PE on oxidative stress in serum**

The activity of the antioxidant enzyme SOD in the AMI group was significantly decreased compared with that in normal controls or sham-operated animals (p < 0.001, Fig. 3A) while the amount
Fig. 1. Qualitative and quantitative analyses of PE. (A) RP-HPLC spectrum of pure paeoniflorin; (B) RP-HPLC spectrum of PE; (C) UV spectrum of pure paeoniflorin, and (D) UV spectrum of PE. The HPLC analysis was performed using a ZORBAX Extend C18 reverse phase column (4.6 mm × 150 mm, 5 μm) with acetonitrile–water (1:4) as mobile phase at a rate of 1 ml/min at room temperature. The UV scanning wavelength was at 200–350 nm.

Fig. 2. AMI-induced changes in cytokines (TNF-alpha and IL-10) in serum, and the protective effects of PE and isosorbide dinitrate. Levels of TNF-α and IL-10 were determined with ABC-ELISA kits as described in the Materials and methods section. (A) TNF-alpha and (B) IL-10. Values are mean ± SEM, n = 8. Abbreviations: Con, normal control group; Sham, Sham-operated group; AMI, acute myocardial infarction group; PE-L and PE-H for peony extract low- and high-dose-treated groups, respectively; and isosorbide, isosorbide dinitrate-treated group. *p < 0.05, **p < 0.01 vs. Con; †p < 0.05, ††p < 0.01 vs. AMI.

The effect of PE on blood coagulation

TT in AMI rats decreased significantly when compared with that of normal control or sham-operated animals (p < 0.001). All treatments, PE low dose, PE high dose and isosorbide dinitrate, prevented the AMI-induced decrease in TT (Table 1).

APTT in AMI rats increased significantly when compared with that of normal control animals (p < 0.001), but not significantly when compared with that of sham-operated animals. Treatments

Fig. 3. AMI-induced changes in oxidative stress indexes (SOD and MDA) in serum, and the protective effects of PE and isosorbide dinitrate. SOD activity was measured with a xanthine oxidase system and MDA levels were measured with the thiobarbituric acid assay. (A) SOD and (B) MDA. Values are mean ± SEM, n > 5. *p < 0.05, **p < 0.01 vs. Con; †p < 0.05, ††p < 0.01 vs. AMI.
The effect of PE on cardiomyocyte apoptosis

Cardiomyocyte apoptosis was determined by flow cytometry. Fig. 4A shows representative flow cytometric two-dimensional plots for each group and Fig. 4B gives the quantitative results. Compared with normal controls, sham-operation caused a slight, and AMI, a significant increase in the early-stage apoptotic ratio (ratio of early-stage apoptotic cells over total cells, p<0.001; Fig. 4A and B). Treatment with PE low dose, PE high dose or isosorbide dinitrate significantly prevented the AMI-induced increase in the early-stage apoptotic ratio in cardiomyocytes.

The effect of PE on caspase-3 activity in cardiomyocytes

Compared with that in normal control and sham-operated animals, the caspase-3 activity increased significantly in the AMI animals (p<0.001). Treatments with PE low dose and isosorbide dinitrate significantly reduced the AMI-induced increase in caspase-3 activity, but treatment with PE high dose not only did not cause reduction, but even increased its activity slightly (Table 3).

Table 1
AMI-induced changes in blood anticoagulation indexes (TT, APTT and PT) in serum, and the protective effects of PE and isosorbide dinitrate.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>TT (s) Sample</th>
<th>PT (s) Sample</th>
<th>APTT (s) Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>6</td>
<td>25.47 ± 4.58</td>
<td>19.32 ± 3.65</td>
<td>30.36 ± 1.64</td>
</tr>
<tr>
<td>Sham</td>
<td>5</td>
<td>23.85 ± 5.93</td>
<td>19.45 ± 2.53</td>
<td>25.60 ± 0.96</td>
</tr>
<tr>
<td>AMI</td>
<td>9</td>
<td>13.46 ± 2.37**</td>
<td>27.65 ± 5.16**</td>
<td>23.77 ± 2.03**</td>
</tr>
<tr>
<td>PE-L</td>
<td>7</td>
<td>17.48 ± 4.22**</td>
<td>25.79 ± 1.70</td>
<td>24.39 ± 3.10</td>
</tr>
<tr>
<td>PE-H</td>
<td>9</td>
<td>23.93 ± 1.27**</td>
<td>21.74 ± 2.10</td>
<td>27.78 ± 1.74**</td>
</tr>
<tr>
<td>Isosorb</td>
<td>10</td>
<td>21.58 ± 2.85**</td>
<td>20.10 ± 3.71</td>
<td>33.50 ± 1.68**</td>
</tr>
</tbody>
</table>

Abbreviations: Con for control group; Sham for sham-operated group; AMI for AMI group; PE-L for low dose PE; PE-H for high dose PE; Isosorb for isosorbide. Values are mean ± SEM, n>5.

* p<0.01 vs. Con; ** p<0.01 vs. AMI.

Table 2
AMI-induced changes in left ventricular function and the protective effects of PE and isosorbide dinitrate.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>BP/LVSP Av. Max (mmHg)</th>
<th>BP/LVSP Av. Mimb (mmHg)</th>
<th>dp/dtmax Av. Max (mmHg/s)</th>
<th>−dp/dtmax Av. Min (mmHg/s)</th>
<th>BP/LVSP Av. Rate (BPM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>11</td>
<td>107 ± 10</td>
<td>-2.72 ± 0.54</td>
<td>4731.88 ± 98.24</td>
<td>-3818.29 ± 94.25</td>
<td>347.84 ± 21.55</td>
</tr>
<tr>
<td>Sham</td>
<td>10</td>
<td>122 ± 15</td>
<td>-3.66 ± 0.43</td>
<td>5853.23 ± 120.45</td>
<td>-4435.57 ± 106.57</td>
<td>375.64 ± 20.12</td>
</tr>
<tr>
<td>AMI</td>
<td>11</td>
<td>107 ± 13</td>
<td>-1.87 ± 0.47</td>
<td>4906.54 ± 118.26</td>
<td>-3858.18 ± 134.25</td>
<td>375.04 ± 15.35</td>
</tr>
<tr>
<td>PE-L</td>
<td>12</td>
<td>113 ± 14</td>
<td>-2.15 ± 0.25</td>
<td>5367.24 ± 94.58</td>
<td>-3960.27 ± 98.87</td>
<td>374.41 ± 19.36</td>
</tr>
<tr>
<td>PE-H</td>
<td>11</td>
<td>111 ± 9</td>
<td>-3.00 ± 0.66</td>
<td>5218.37 ± 120.36</td>
<td>-3916.51 ± 122.65</td>
<td>371.93 ± 17.24</td>
</tr>
<tr>
<td>Isosorb</td>
<td>12</td>
<td>107 ± 11</td>
<td>-1.54 ± 0.51</td>
<td>4989.09 ± 115.40</td>
<td>-3765.66 ± 112.54</td>
<td>377.89 ± 16.87</td>
</tr>
</tbody>
</table>

Abbreviations: Con for control group; Sham for sham-operated group; AMI for AMI group; PE-L for low dose PE; PE-H for high dose PE; Isosorb for isosorbide; Av. Max for Average maximum values; Av. Min for Average minimum values, and Av. Rate for average rate.

* p<0.001.
** p<0.05 vs. control group (Con).
*** p<0.001 vs. AMI group.

with PE high dose and isosorbide dinitrate, but not PE low dose, significantly prevented the AMI-induced decrease in APTT (Table 1).

PT in AMI rats increased significantly when compared with normal control or sham-operated animals (p<0.001). The PE high dose and isosorbide dinitrate treatments, but not PE low dose, prevented the AMI-induced increase in PT (Table 1).

The effect of PE on cardiac function

The effects of PE on cardiac function are summarized in Table 2. Compared with that of normal control or sham-operated animals, LVEDP increased significantly in AMI animals (p<0.001). The treatments decreased the AMI-induced level of LVEDP by 40.0% (PE low dose), 65.1% (PE high dose), and 18.3% (isosorbide dinitrate). All these reductions were significant (p<0.001).

The effect of PE on caspase-3 activity in cardiomyocytes

Compared with that in normal control and sham-operated animals, the caspase-3 activity increased significantly in the AMI animals (p<0.001). Treatments with PE low dose and isosorbide dinitrate significantly reduced the AMI-induced increase in caspase-3 activity, but treatment with PE high dose not only did not cause reduction, but even increased its activity slightly (Table 3).
Table 3
AMI-induced changes in caspase-3 activity, and the protective effects of PE and isosorbide dinitrate.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Caspase-3 activity (U/μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>7</td>
<td>581.18 ± 106.46</td>
</tr>
<tr>
<td>Sham</td>
<td>8</td>
<td>574.06 ± 86.39</td>
</tr>
<tr>
<td>AMI</td>
<td>9</td>
<td>685.98 ± 50.10*</td>
</tr>
<tr>
<td>PE-L</td>
<td>7</td>
<td>576.55 ± 95.91*</td>
</tr>
<tr>
<td>PE-H</td>
<td>8</td>
<td>703.24 ± 63.93</td>
</tr>
<tr>
<td>Isosorb</td>
<td>7</td>
<td>536.80 ± 63.12</td>
</tr>
</tbody>
</table>

Abbreviations: Con for control group; Sham for sham-operated group; AMI for AMI group; PE-L for low dose PE; PE-H for high dose PE; Isosorb for isosorbide. Values are mean ± SEM, n > 7.

**p < 0.01 vs. Con.
ˆˆp < 0.01 vs. AMI.

The effect of PE on apoptosis-related gene expression

Expression levels of the apoptosis-related genes bcl-2, bax, and fas were detected with electrophoresis and their band densities were analyzed with Bandscan 5.0 software. Fig. 5A shows a few representative electrophoretic images and Fig. 5B gives quantitative results of the band densities. Compared with those in normal control or sham-operated animals, the expressions of bcl-2, bax and fas were increased in the AMI animals. Treatments with PE low dose, PE high dose, or isosorbide dinitrate prevented the AMI-induced increase in the expressions of these genes. The bcl-2:bax ratio in the AMI group was 3.94 times that in the sham-operated group. The bcl-2:bax ratios in the PE low dose and PE high dose groups were 1.24 and 1.37 times that in the AMI group, respectively.

**Fig. 5.** AMI-induced changes in apoptosis-related genes (bcl-2, bax and fas) and the protective effects of PE and isosorbide dinitrate. The gene expressions were determined by PCR and the bands were analyzed with Bandscan 5.0 following agarose gel electrophoresis. (A) Representative electrophoretic images for actin, bcl-2, bax, and fas. (B) Quantitative results derived from the band densities of bcl-2, bax, fas, and actin.

The effect of PE on serum myocardial enzymes

The effects of PE on myocardial enzymes are summarized in Table 4. Compared with those in normal control or sham-operated animals, the activities of all myocardial enzymes measured, including LDH, GOT, α-HBDH, CK, and CK-MB, were significantly increased in AMI rats. The treatments with PE low dose, PE high dose and isosorbide dinitrate all inhibited the AMI-induced increase in the activities of these enzymes. PE had a dose-dependent effect because the high dose PE showed a more significant inhibition than the low PE dose. The effects of the PE high dose were comparable to those of the clinically used drug isosorbide dinitrate.
Table 4
AMI-induced changes in activities of myocardial enzymes and the protective effects of PE and isosorbide dinitrate.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>LDH-L (U/l)</th>
<th>GOT (U/l)</th>
<th>α-HBDH (U/l)</th>
<th>CK (U/l)</th>
<th>CK-MB (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>8</td>
<td>629.47 ± 74.24</td>
<td>200.23 ± 15.07</td>
<td>180.81 ± 12.25</td>
<td>853.01 ± 84.12</td>
<td>978.13 ± 92.04</td>
</tr>
<tr>
<td>Sham</td>
<td>9</td>
<td>875.46 ± 80.19</td>
<td>209.30 ± 17.28</td>
<td>223.62 ± 13.21</td>
<td>1369.86 ± 95.68</td>
<td>1019.11 ± 89.45</td>
</tr>
<tr>
<td>AMI</td>
<td>11</td>
<td>1121.25 ± 77.51</td>
<td>248.02 ± 14.24</td>
<td>284.49 ± 10.56</td>
<td>1114.24 ± 96.57</td>
<td>1287.70 ± 100.56</td>
</tr>
<tr>
<td>PE-L</td>
<td>9</td>
<td>1226.67 ± 90.97</td>
<td>225.15 ± 11.65</td>
<td>250.38 ± 16.11</td>
<td>1157.77 ± 91.30</td>
<td>1216.74 ± 94.18</td>
</tr>
<tr>
<td>PE-H</td>
<td>9</td>
<td>988.46 ± 81.55</td>
<td>212.87 ± 10.58</td>
<td>204.27 ± 12.14</td>
<td>842.53 ± 76.35</td>
<td>916.91 ± 84.51</td>
</tr>
<tr>
<td>Isosorb</td>
<td>10</td>
<td>725.44 ± 60.22</td>
<td>194.32 ± 9.54</td>
<td>278.71 ± 16.35</td>
<td>608.04 ± 80.23</td>
<td>1070.54 ± 91.27</td>
</tr>
</tbody>
</table>

Abbreviations: Con for control group; Sham for sham-operated group; AMI for AMI group; PE-L for low dose PE; PE-H for high dose PE; Isosorb for isosorbide; LDH for lactate dehydrogenase; GOT for glutamic oxaloacetic transaminase; C. α-HBDH for alpha-hydroxybutyric dehydrogenase; CK for creatine kinase; and CK-MB for creatine kinase isoenzyme MB. Values are mean ± SEM, n ≥ 8.

Discussion

Injury due to myocardial ischemia occurs following inhibition of the aerobic oxidation of glucose, augmentation of anaerobic glycolysis and accumulation of lactic acid dehydrogenase. Meanwhile, reduction of ATP production, disruption of ionic gradients and degradation of membrane stability can lead to out-leakage of enzymes normally residing within cardiomyocytes. Consequently, the content of myocardial enzymes in blood increases, so changes in serum myocardial enzymes are considered to be a measure of impairment produced by myocardial ischemia. In this study, the activity of CK-MB in AMI rats was higher than in control rats. PE low dose had no significant effect compared with the AMI group (p = 0.124). High PE dosing had a significant protective effect as evidenced by a significant decrease in CK-MB activity compared with the AMI group (p < 0.001). Similarly, the activities of LDH, α-HBDH, GOT and CK in AMI were increased significantly in the AMI group, compared with normal controls (p < 0.001). PE dose-dependently protected against the increases in LDH, α-HBDH, GOT and CK activities in AMI rats. These results suggest that the protective effects against AMI damage may be produced by reducing the activity of these enzymes, elevating cardiomyocyte membrane stability and by decreasing out-leakage of enzymes.

TNF-α and IL-10 have many biological effects. The main function of TNF-α is mediating inflammatory reactions. It can cause dysfunction in tissues and organs by inducing the death of cardiomyocytes, apoptosis, dilatation of the left ventricle, and thinning of the left ventricular wall. IL-10 is critical for inhibition of immunity. IL-10 can inhibit the proliferation of immunocytes by releasing cytokines and reducing the level of TNF-α. IL-10 has a protective function in cardiac muscle. Myocardial ischemia and oxidative stress can elevate the levels of TNF-α and IL-10. From our results, the contents of TNF-α and IL-10 were elevated in AMI rats compared with normal control or sham-operated rats (p < 0.001). That suggests that TNF-α and IL-10 are involved in the remodeling of cardiac muscle. PE effectively protected against the elevation of IL-10 and TNF-α. These results suggest that PE regulates AMI-induced inflammation by reducing the release of cytokines.

MDA, a product of lipid peroxidation, can interfere with cell proliferation and protein expression. Products of lipid peroxidation react with the free amino groups of proteins and nucleic acids to form Schiff bases that crosslink biological macromolecules (Esterbauer, 1993; Long et al., 2009). Consequently, membrane integrity of myocardial cells is degraded, resulting in inhibition of the physiological functions of the heart, serious arrhythmias and cellular necrosis. SOD is a very important antioxidant enzyme that has the functions of scavenging superoxide anion, preventing cellular damage and maintaining the balance of oxidation and anti-oxidation. Our results showed that AMI induced an increase in MDA levels and a decrease in SOD activity, suggesting an increase in oxidative stress and revealing an imbalance between the production and scavenging of oxygen free radicals in rats subjected to myocardial ischemia. PE decreased the production of MDA and increased the activity of SOD, suggesting that PE acts as an antioxidant that reinforces functioning of the system that scavenges endogenous oxygen free radicals so as to inhibit oxidative stress (such as lipid peroxidation) and consequently protect cardiomyocytes from oxidative damage.

PT is the time needed for plasma to clot under conditions mimicking exogenous blood coagulation. PT is used for detecting the abnormality of exogenous coagulation factors and is the most commonly used measurement of blood coagulation function. APTT is an important measurement for screening thrombus and thrombosis. It can detect propeptide-releasing enzymes and plasma coagulation factors, including factors VIII, IX, X, XI. TT (thrombin time) is an index coupled with PT. Thrombin can up-regulate the expression of vascular endothelial growth factor (VEGF) receptor mRNA to enhance angiogenesis. TT reflects the ability of forming plasma fibrinogen to fibrin (Elg et al., 1999). Thrombin can induce blood clotting dysfunction and shorten PT, APTT and TT. An in vitro study has shown that PE may extend PT, APTT and TT, and improve coagulation function [9]. In the present study, we showed that TT was significantly shortened in AMI rats and PE dose-dependently prolonged TT. It seems sham-operation caused a significant decrease in APTT, which AMI slightly decreased further. Nevertheless, PE showed a dose-dependent prolongation of APTT in AMI rats. These results suggest that PE inhibits thrombin and fibrin polymerization and endogenous blood coagulation. But the change in PT was not consistent with the changes in TT and APTT. The reason is unknown but warrants further study.

Haemodynamics is a kind of method to study cardiovascular function. Myotility and haemodynamics can be evaluated through many kinds of indexes, and the changes in LVEDP can be used to measure ventricular metergasis. The increase in LVEDP directly represents the increase in cardiac preload and also indirectly reflects the impairment of cardiac diastolic function. Our results showed that the LVEDP of AMI rats increased significantly compared with normal controls (p < 0.001) and PE effectively prevented the APTT-induced increase (p < 0.001). These results suggest that PE may inhibit collagen hyperplasia, lessen the interstitial fibrosis of cardiac muscle, and increase the compliance of cardiac muscle so as to improve the heart’s diastolic function.

Apoptosis is programmed cell death, the culmination of a series of gene expression changes and breakage of DNA. Studies have demonstrated that apoptosis in cardiomyocytes occurs in acute cardiovascular diseases, such as myocardial ischemia and myocardial infarction (Olivetti et al., 1996), and in long-term ischemia and ischemia-reperfusion (Kajstura et al., 1996). We observed that, compared to normal rats, the number of apoptotic cardiomyocytes increased in AMI rats and PE protected against the increase.
This confirms that acute myocardial infarction can induce apoptosis and suggests that PE is able to protect against it. Apoptosis includes the cutting of DNA by endonucleases, phosphatidylycerine turnover and caspase activation. Therefore, we have measured caspase-3 activity and found that, compared to controls, the activity of caspase-3 in AMI animals increased significantly; PE prevented this increase, suggesting that PE may prevent AMI-induced cardiac dysfunction by inhibiting apoptosis and deactivating caspas.

Apoptosis is regulated by both induction and repression of gene expression. Bcl-2 has an anti-apoptotic function and promotes cell survival. Binding of Bax with Bcl-2 inhibits apoptosis, while formation of Bax dimers induces it. Bax dimers form when Bax is overexpressed. Generally, once the pro-apoptotic process is activated, expression of related anti-apoptotic proteins will increase. The results of this study revealed that Bcl-2 increased and the Bcl-2/Bax ratio decreased in AMI rats and that PE was protective. Fas is an important cell surface receptor in the caspase pathway. Our results show that Fas expression was up-regulated in AMI. Low dose and high dose PE had no obvious protection. These results suggest that PE may prevent cardiomyocyte apoptosis by regulating the bcl-2 and box genes, but not the fas gene.

In summary, PE protected cardiac muscle from the following: damage to cytomembranes, outbreakage of myocardial enzymes, the decreases in antioxidant defenses and blood anticoagulating ability, and the increases in TNF-α and IL-10 in AMI rats. Some of the protective effects of PE were even better than the clinically used drug isosorbide dinitrate. Mechanistic study demonstrated that PE’s protective effect may be mediated by regulation of the apoptosis-related genes Bcl-2 and Bax and a critical apoptotic enzyme, caspase-3. These results suggest that PE may be an effective protective agent for preventing and treating AMI and other cardiovascular diseases. Though paeoniflorin is proposed to be the major active component of PE, it is still necessary to determine the contents of other minor components and compare the effects of paeoniflorin and other components on various biomarkers and pathways for a better understanding of the protective mechanisms of PE in AMI in the future studies.

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References