Effects of dl-praeruptorin A on interleukin-6 level and Fas, bax, bcl-2 protein expression in ischemia-reperfusion myocardium

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KEY WORDS dl-praeruptorin A; myocardium; ischemia; reperfusion injury; apoptosis; interleukin-6

ABSTRACT

AIM: To investigate the effects of dl-praeruptorin (Pd-Ia) on interleukin-6 (IL-6) level and apoptosis-related protein expression in ischemia-reperfusion myocardium. METHODS: Left anterior descending coronary artery was subjected to 30 min ischemia followed by 120 min reperfusion in open-chest anesthetized rats. Serum IL-6 level was measured by radioimmunoassay. Apoptosis-related protein Fas, bax, and bcl-2 expression was detected by immunohistochemistry and computer image analysis system. Infiltration of neutrophils was observed using Hematoxylin-Eosin staining under optical microscope. RESULTS: Pd-Ia 2.0 mg·kg⁻¹ iv lowered serum IL-6 level and Fas, bax, bcl-2 expression under conditions with hypotension and without changes on heart rate, but increased the ratio of bcl-2/bax. There existed a close linearity and positive correlation between IL-6 level and Fas, bax, bcl-2 expression. Whereas, the infiltration of neutrophils was mild. CONCLUSION: Pd-Ia elicits a novel target in the therapeutic prevention of postischemic cardiomyocyte death. The reason might be associated with modulating the expression of some immediate-early genes including IL-6, Fas, bax, and bcl-2 in ischemia-reperfusion myocardium.

INTRODUCTION

A number of studies have shown that ischemia-reperfusion induce inflammatory cytokine gene expres-
contractility and induction of cardiomyocyte apoptosis\(^9\). However, the molecular mechanism of controlling and regulating the expression of these immediately early genes during MIR has remained unclear. As cardiomyocyte apoptosis is one of the major contributors to the pathogenesis, accordingly, prevention of it may be a reasonable therapeutic strategy.

In our previous studies, a pyranocoumarin component 4,8,13,17-dl-praeuertorin (Pd-Ia) isolated from Baihua Qianhu, a traditional Chinese medicine (Peucedanum praeruptorum Dunn), as a Ca\(^{2+}\)-influx blocker\(^10-12\) and K\(^+\)-channel opener\(^13\), displays myocardial protection\(^14-16\).

In the present study, we mainly investigated the effects of Pd-Ia on serum IL-6 level and Fas, bax, bcl-2 protein expression in ischemia-reperfusion myocardium of rats, in order to further explore the activity and mechanism of Pd-Ia.

MATERIALS AND METHODS

**Drugs and reagents** Pd-Ia was presented friendly by Prof OKUYAMA T (Department of Pharmacognosy and Phytochemistry, Meiji College of Pharmacy, Tokyo 154, Japan); \(^{125}\)I-interleukin-6 radioimmunization kit was produced by 301 Radioimmunization Research Center, Beijing, China; Rabbit anti-rat monoclonal antibodies (including Fas, bax, and bcl-2) were prepared by Santa Cruz Biological Technology Co; Instant-type SABC (Strept-Avidin-Biotin-enzyme Complex) immunohistochemistry kit was provided by Wuhan Boster Biological Technology Co.

**Experimental protocol** Forty-seven healthy male Wistar rats of both sexes, weighing 274 g±16 g were randomly divided into 7 subgroups (n=6-7). Group I, sham operation control; Group II, operation control; Group III, solvent control (PEG400); Group IV, positive control (nifedipine); Group V, Pd-Ia 0.5 mg·kg\(^{-1}\); Group VI, Pd-Ia 1.0 mg·kg\(^{-1}\); Group VII, Pd-Ia 2.0 mg·kg\(^{-1}\).

Under urethane-chloralose anaesthesia, open-chest, and artificial ventilation, left anterior descending coronary artery of rat was dissected. A 5-0 silk suture was passed around the vessel and an occlusive snare was placed around it. Thirty minutes ischemia followed by 120 min reperfusion was produced as reported previously\(^14\). Mean carotid arterial pressure (BP) and heart rate (HR) were measured using 8-channel recorder (RM-6000, Nihon Kohden).

A bolus of Pd-Ia was injected iv while onset of reperfusion. Blood sample was drawn out from right ventricular chamber at 120 min after reperfusion. Serum IL-6 level was determined by radioimmunoassay. Partial myocardium from area subjected to MIR was scissored snap-frozen, and sliced by freezing microtome. Fas, bax, and bcl-2 expression was surveyed by immunohistochemistry and computer image analysis system\(^17\). Chromogen reaction was performed with 3’,3-diaminobenzidine 0.5 g/L (25 \(\pm\)1 \(\mu\)g, 10 min), counterstained in hematoxylin and observed under light microscopy. Brown staining in epicyte or cytoplasm was evaluated as positive expression, which was examined by calculating the ratio of mean optic density and area in positive stain, namely, positive expressive index (PEI).

Infiltration of neutrophil was examined by Hematoxylin-Eosin staining under optical microscope.

**Statistical analysis** All data was expressed as mean±SD. Unpaired student’s \(t\)-test was used to assess statistical significance of differences between Pd-Ia and various related control groups. Comparison of parameters before and after administration of drugs was performed using one-way ANOVA analysis of variance. \(P<0.05\) was considered to be significant.

RESULTS

**Effects of Pd-Ia on serum IL-6 level** Pd-Ia decreased serum IL-6 level at higher dose of 2.0 mg/kg markedly [(116±11) ng/L vs (153±15) ng/L in solvent control, \(P<0.05\)]. This decrease was similar to that of nifedipine. IL-6 level in operation control was higher than that of sham control, suggesting that MIR induced the overexpression of IL-6, but no significant change was found in solvent control (Tab 1).

**Effects of Pd-Ia on Fas, bax, and bcl-2 expression** Pd-Ia inhibited Fas, bax, and bcl-2 expression (Fig 1). At the dose of Pd-Ia 2.0 mg·kg\(^{-1}\), PEI of Fas, bax, and bcl-2 were decreased significantly (3.4 %±
Tab 1. Effects of Pd-Ia on serum IL-6 level and Fas, bax, bcl-2 expression in ischemia-reperfusion myocardium of rats. PEI: positive expressive index; Sham: sham control; Oper: operation control; Sol: solvent control; Nif: nifedipine control. Mean±SD. \( ^* P<0.05, ^{**} P<0.01 \) vs solvent group. \( ^{**} P<0.01 \) vs sham group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose/mg kg(^{-1})</th>
<th>n</th>
<th>IL-6/ng L(^{-1})</th>
<th>Fas</th>
<th>PEI/%</th>
<th>bcl-2</th>
<th>bcl-2/bax</th>
</tr>
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<tbody>
<tr>
<td>Sham</td>
<td>-</td>
<td>7</td>
<td>122±6</td>
<td>1.9±0.5(^*)</td>
<td>1.4±0.3(^*)</td>
<td>1.8±0.5(^*)</td>
<td>1.35±0.11(^*)</td>
</tr>
<tr>
<td>Oper</td>
<td>-</td>
<td>6</td>
<td>152±18</td>
<td>7.0±2.4(^*)</td>
<td>5.9±1.4(^*)</td>
<td>5.7±1.0(^*)</td>
<td>0.96±0.11(^*)</td>
</tr>
<tr>
<td>Sol</td>
<td>-</td>
<td>7</td>
<td>153±15</td>
<td>6.7±1.5(^*)</td>
<td>6.0±1.0(^*)</td>
<td>5.4±0.9(^*)</td>
<td>0.92±0.09(^*)</td>
</tr>
<tr>
<td>Nif</td>
<td>5.0</td>
<td>6</td>
<td>124±19(^*)</td>
<td>3.6±0.6(^*)</td>
<td>1.8±0.4(^*)</td>
<td>3.6±0.6(^*)</td>
<td>2.05±0.22(^*)</td>
</tr>
<tr>
<td>Pd-Ia</td>
<td>0.5</td>
<td>7</td>
<td>129±18</td>
<td>3.8±0.7(^*)</td>
<td>3.5±0.4(^*)</td>
<td>4.2±1.0</td>
<td>1.16±0.17</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>7</td>
<td>120±16(^*)</td>
<td>3.5±0.7(^*)</td>
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<td>3.4±0.9(^*)</td>
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</tr>
<tr>
<td></td>
<td>2.0</td>
<td>7</td>
<td>116±11(^*)</td>
<td>3.4±0.9(^*)</td>
<td>2.1±0.4(^*)</td>
<td>2.9±0.7(^*)</td>
<td>1.42±0.06(^*)</td>
</tr>
</tbody>
</table>

Fig 1. Effects of Pd-Ia on Fas (b), bax (c), and bcl-2 (d) protein expression in left ventricular myocardium subjected to 30 min ischemia followed by 120 min reperfusion in open-chest anesthetized rats treated by a bolus injection intravenously of solvent (PEG 400) solution 1 mL/kg (a) or dl-praeeruptorin A 2.0 mg/kg (b, c, and d) immediately before reperfusion. Epicyte or cytoplasm brown staining indicates positive expression. \( \times 200 \).

0.9 %, 2.1 %±0.4 %, and 2.9 %±0.7 % vs 6.7 %±1.5 %, 6.0 %±1.0 %, and 5.4 %±0.9 % in solvent control, \( P<0.01 \), in contrast, it increased the ratio of bcl-2/bax (1.42±0.06 vs 0.92±0.09 in solvent control, \( P<0.01 \)). The attenuation was a little similar but weak to that of nifedipine. Fas, bax, bcl-2 expression in operation control was markedly higher than sham control (\( P<0.01 \)), whereas the ratio of bcl-2/bax was lower (\( P<0.05 \), Tab 1), indicating that MIR assuredly lead to apoptosis-related protein overexpression.

**Correlation analysis between serum IL-6 level and Fas, bax, and bcl-2 expression** There existed a
close linearity and positive correlation between serum IL-6 level and Fas, bax, bcl-2 expression (Fig 2). The correlation coefficients (r) were 0.8324, 0.7429, and 0.7423 (P<0.01), respectively.

Effects of Pd-Ia on infiltration of inflammatory cells The infiltration of neutrophils in Pd-Ia group displayed mild, and was not seen in sham control, whereas most appeared in operation and solvent controls (Fig 3).

Effects of Pd-Ia on BP and HR Pd-Ia decreased BP significantly, and the duration sustained over 60 min (Tab 2). The BP at maximal dose reached from 12.6 kPa±0.7 kPa to 9.4 kPa±1.2 kPa at 30 min after administration (25 %±6 % decreased, P<0.01 vs solvent group), but the potency was weaker than that of nifedipine (41 %±11 % decreased, P<0.05 vs nifedipine group). Furthermore, Pd-Ia had no influence on HR.

DISCUSSION

Recent basic experimental and clinical evidence suggest that brief coronary occlusion followed by reperfusion leads to reversible myocardial dysfunction[18], whereas cardiomyocyte death during MIR is partially mediated by apoptosis[19,20]. In the present study, we have demonstrated that Pd-Ia reduced serum IL-6 level and Fas, bax, bcl-2 expression during MIR under conditions with hypotension and without changes on HR in rats. Meanwhile, the action of Pd-Ia was strongly similar but weaker to that of nifedipine, a calcium channel blocker. So we hypothesize that the mechanisms of action of Pd-Ia are probably associated with blockade of Ca$^{2+}$ influx, inhibition of Fas-Fas ligands, and Ca$^{2+}$-
dependence, etc. Those are all capable of abolishing Ca$^{2+}$-overload of cytoplasm evoked by MIR, maintaining balance between intra- and extra-cellular calcium, remaining vasodilatation and stability of mitochondria, finally, leading to lower the occurrence of cardiomyocyte apoptosis. Thus, it is preferable to other vasodilators or calcium antagonists in cardiohemodynamic modulation and cardioprotection during MIR.

Secondly, the fact that Pd-Ia reduced Fas, bax, and bcl-2 expression also involved other factors such as fall of IL-6 level, alleviation of neutrophils infiltration, lowering heart load and myocardial oxygen consumption via indirect amelioration of cardio-hemodynamics, etc.

In conclusion, controlling and regulating the expression of some immediate-early genes including IL-6, Fas, bax, and bcl-2, exhibits a relievable or beneficial effect in ischemia-reperfusion myocardium. Pd-Ia elicits a novel target in the therapeutic prevention of postischemic cardiomyocyte death. It might be as a therapeutic agent to open a promising perspectives in prevention of MIR and cardioprotection.

REFERENCES


Keywords: 前胡甲素, 心肌, 缺血, 再灌注损伤, 细胞凋亡, 白介素-6.