Original article

Cardioprotective effects of low-dose combination therapy with a statin and an angiotensin receptor blocker in a rat myocardial infarction model

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KEYWORDS
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Left ventricular systolic function;
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Summary
Purpose: Statins attenuate angiotensin II-induced myocyte hypertrophy and this might increase the cardioprotective effects of renin—angiotensin system inhibition in the ischemic heart. In this study, we investigated the cardioprotective effects of combination therapy with low-dose simvastatin and low-dose losartan using a rat myocardial infarction model.
Methods: Myocardial infarction was created in rats by left anterior descending artery ligation, and the animals were randomly allocated to one of four groups: control (n = 8), losartan 3 mg/kg/day (n = 8), simvastatin 2 mg/kg/day (n = 8), and losartan 3 mg/kg/day plus simvastatin 2 mg/kg/day (n = 8). Each treatment was started on the day of coronary ligation, and hemodynamics, myocardial blood flow, and infarct size were measured after 28 days.
Results: Blood pressure, heart rate, and left ventricular systolic and end-diastolic pressures were not significantly different comparing the control group with the 3 other treatment groups.

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Introduction

Blockade of the renin–angiotensin system (RAS) by angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) inhibits left ventricular hypertrophy and left ventricular remodeling, ameliorates left ventricular dysfunction [1], and consequently improves long-term prognosis [2] in patients with ischemic as well as non-ischemic heart failure.

The 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors, statins, have been shown to reduce cardiovascular morbidity and mortality due to cholesterol-lowering effects [3]. However, other lipid-independent pleiotropic actions may contribute to cardioprotection induced by statins [4], and this has been documented in experimental studies demonstrating a decrease in oxidative stress, inflammation, inhibition of thrombogenic response, and atherosclerotic plaque formation [5] resulting in attenuation of vascular endothelial dysfunction. The mechanism responsible for the pleiotropic effects of statins involve the inhibition of small GTP-binding proteins, such as Ras, Rho, and Rac, which modulate a wide variety of cellular process [4] including the myocardial response to ischemic injury. We hypothesized that statins might have synergistic cardioprotective effects for renin–angiotensin system inhibition in the ischemic heart. Based on this hypothesis, in this study we investigated the effects of combination therapy with a statin (simvastatin) or an ARB (losartan) using a low-dose of both agents, which might not be effective, on cardiac function, coronary blood flow, and infarct size in a rat myocardial infarction model.

Methods

Study protocol

The left anterior descending coronary artery was ligated 2 mm below the left atrium with a 7–0 prolene suture in 32 adult (8-week-old) male Sprague-Dawley rats (191.9–230.6 g). Rats were randomly allocated to one of 4 treatment groups: control (0.5% CMC-Na solution; n = 8), losartan 3 mg/kg/day (n = 8), simvastatin 2 mg/kg/day (n = 8), or losartan 3 mg/kg/day plus simvastatin 2 mg/kg/day (n = 8). Doses for oral administration were decided to be 10% of commonly used doses, 30 mg/kg/day and 20 mg/kg/day for losartan and simvastatin in the rat experiment, respectively, which yields plasma concentration similar to that seen in patients taking clinical doses. Both agents were suspended in 0.5% CMC-Na solution and were given to rats by gastric gavage once a day. Each treatment was started on the day of onset of myocardial infarction by coronary ligation. Hemodynamic measurements were performed 28 days after the onset of myocardial infarction under barbiturate anesthesia and controlled ventilation. After the measurements, the anesthetized rats were euthanized with a lethal dose of barbiturate, and the hearts were removed to measure myocardial blood flow and infarct size.

Hemodynamic measurement

Under barbiturate anesthesia and controlled ventilation, a catheter was inserted into the right carotid artery to measure systolic (SBP) and diastolic blood pressure (DBP) and heart rate (HR). Then, the catheter tip was advanced into the left ventricle to record left ventricular systolic (LVSP) and end-diastolic pressures (LVEDP). Finally, left ventricular pressure was differentiated and the peak positive first derivative of left ventricular pressure (peak LV dP/dt) was measured.

Myocardial blood flow measurement

In 4 rats from each group, myocardial blood flow was measured using fluorescent microspheres. The microspheres were injected into the left ventricle through the catheter. Another catheter was inserted into the abdominal aorta via the femoral artery and a reference blood sample was withdrawn at a rate of 0.84 mL/min. After euthanasia, the left ventricle was removed and weighed. The left ventricular tissue was digested with 4 M potassium hydroxide containing 2% dimethyl formamide was added to extract the dye in the solution. The solution was centrifuged at 4700 rpm for 5 min. Finally, the supernatant was collected to measure the absorbance and the optical density (OD) was calculated. The reference blood sample was similarly treated and myocardial blood flow was calculated using a standard formula [tissue OD × reference blood withdrawal rate/tissue weight (g) × reference blood OD].

Determination of infarct size

In 4 rats of each group, the infarct size was determined. After euthanasia, the whole heart was excised and the right and left ventricles were dissected. The weights of the whole heart, the right ventricle and the left ventricle were measured. Then, the left ventricle was sliced into 5
Effect of statin on myocardial infarction

Table 1  Global parameters at the 28th day in each treatment group.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=8)</th>
<th>Losartan (n=8)</th>
<th>Simvastatin (n=8)</th>
<th>Losartan+Simvastatin (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>125 ± 7</td>
<td>127 ± 4</td>
<td>126 ± 5</td>
<td>141 ± 7</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>104 ± 5</td>
<td>105 ± 3</td>
<td>101 ± 3</td>
<td>112 ± 5</td>
</tr>
<tr>
<td>HR (/min)</td>
<td>403 ± 15</td>
<td>441 ± 6</td>
<td>406 ± 12</td>
<td>435 ± 16</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>126 ± 5</td>
<td>129 ± 4</td>
<td>128 ± 6</td>
<td>126 ± 5</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>10.0 ± 1.1</td>
<td>6.8 ± 1.2</td>
<td>10.2 ± 2.5</td>
<td>6.9 ± 0.6</td>
</tr>
<tr>
<td>Peak LV dP/dt (mmHg/sec)</td>
<td>7229 ± 408</td>
<td>8263 ± 395</td>
<td>7375 ± 646</td>
<td>9071 ± 528*</td>
</tr>
<tr>
<td>Whole heart weight (g)</td>
<td>1.280 ± 0.036</td>
<td>1.322 ± 0.053</td>
<td>1.343 ± 0.066</td>
<td>1.281 ± 0.047</td>
</tr>
<tr>
<td>Ventricular weight (g)</td>
<td>1.122 ± 0.023</td>
<td>1.146 ± 0.036</td>
<td>1.155 ± 0.036</td>
<td>1.114 ± 0.030</td>
</tr>
<tr>
<td>Left ventricular weight (g)</td>
<td>0.798 ± 0.019</td>
<td>0.816 ± 0.016</td>
<td>0.806 ± 0.015</td>
<td>0.765 ± 0.021</td>
</tr>
<tr>
<td>Myocardial blood flow (mL/min/g)</td>
<td>3.87 ± 0.89 (n=4)</td>
<td>3.96 ± 0.54 (n=4)</td>
<td>3.79 ± 0.47 (n=4)</td>
<td>3.83 ± 0.73 (n=4)</td>
</tr>
<tr>
<td>Infarct size (%)</td>
<td>29.0 ± 1.6 (n=4)</td>
<td>29.8 ± 2.9 (n=4)</td>
<td>26.7 ± 3.5 (n=4)</td>
<td>27.4 ± 2.8 (n=4)</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end diastolic pressure.

*p < 0.05 vs control.

transverse sections. After weighing each slice, 4 of the 5 sections were stained with 1% triphenyl tetrazolium chloride (TTC) in a phosphate buffer to detect infarcted tissue. The infarct area and left ventricular area were measured using specific image analysis software (WinROOF ver. 3.1; Mitani, Tokyo, Japan). The weight of the infarcted tissue in each section was calculated as the infarct area/left ventricular area × section weight. Infarct size (%) was calculated as the weight of all infarcted tissue/left ventricular weight.

Statistical analysis

Data are expressed as the mean ± SE. Normality of the distribution of variables was assessed using a Bartlett test. If the data were normally distributed, comparisons of each treatment group with the control group were performed using the parametric Dunnett test. If the data were not normally distributed, the groups were compared using a nonparametric Dunnett test. A value of p < 0.05 was considered to be statistically significant.

Results

All of the rats survived for 28 days after the onset of myocardial infarction. Global parameters measured on the 28th day of each treatment are shown in Table 1 and Fig. 1. Compared to control group treated with 0.5% CMC-Na+ solution, SBP, DBP, HR, LVSP, and LVEDP were not significantly different in the groups treated with losartan alone, simvastatin alone, and losartan plus simvastatin (Table 1). The peak LV dP/dt was equivalent between the control group and the groups treated with losartan alone and simvastatin alone. However,

Figure 1  Peak positive first derivative of left ventricular pressure (peak LV dP/dt) at the 28th day in each treatment group. The peak LV dP/dt was similar in the groups of losartan alone and simvastatin alone to the control group but was greater in the losartan plus simvastatin group, compared to the control group.
the peak LV $dP/dt$ was significantly greater in the losartan plus simvastatin group than in the control group ($9071 \pm 528$ vs $7229 \pm 408$ mmHg/s, $p < 0.05$) (Fig. 1). Myocardial blood flow was equivalent comparing the control group with the groups treated with losartan alone, simvastatin alone, or losartan plus simvastatin. Furthermore, the whole heart weight, ventricular weight, left ventricular weight, and infarct size (%) were also equivalent comparing the control group with the groups treated with losartan alone, simvastatin alone, or losartan plus simvastatin (Table 1 and Fig. 2).

Discussion

The major finding of our study is that treatment with losartan 3 mg/kg/day plus simvastatin 2 mg/kg/day for 28 days showed an improvement in peak LV $dP/dt$ in a rat myocardial infarction model, although each monotherapy did not show such an effect. Doses of losartan and simvastatin were 10% of commonly used doses in the rat model, 30 mg/kg/day and 20 mg/kg/day, respectively, which yields plasma concentration corresponding to clinical doses. These results suggest that low-dose statins might have cardioprotective effects when given in combination with low-dose ARBs in the ischemic heart.

It has been postulated that inhibition of the renin-angiotensin system produces beneficial effects on cardiac function in ischemic as well as non-ischemic heart failure. ACEIs, ARBs, or both have been demonstrated to increase coronary blood flow and to reduce infarct size in canine myocardial ischemia models via a bradykinin-dependent mechanism [6]. Myocardial fibrosis is a major feature of left ventricular remodeling after myocardial infarction, which is mainly driven by angiotensin II [7,8]. In fact, ACEIs and ARBs have been shown to inhibit cardiac remodeling [8]. Furthermore, it has recently been demonstrated that statins also have cardioprotective as well as vascular protective effects that are independent of their lipid-lowering activity [9]. Acute and chronic statin treatment improves post-ischemic left ventricular contractile dysfunction in the isolated rat heart [10]. Statins also reduce infarct size in acute myocardial infarction or in an ischemia/reperfusion model [11]. Bao et al. demonstrated that the infarct size reduction by statins resulted from the pleiotropic effect represented by increased nitric oxide production [12]. Activation of phosphatidylinositol 3 kinase (PI3K)/Akt by statins may exert a preconditioning-like effect to limit infarct size [13]. In addition, statins may modulate left ventricular remodeling through effects on matrix metalloproteinases [14,15]. Improvement of cardiac function by short-term statin therapy has also been documented in the clinical setting [16,17].

Combined effects of statins and ARBs on cardiovascular systems have been previously reported. Lee et al. demonstrated additive effects of pravastatin and olmesartan on reduced left ventricular remodeling in rat myocardial infarction model [18]. Yamamoto et al. reported that pravastatin enhanced beneficial effects of olmesartan on vascular injury in salt-sensitive hypertensive rats [19]. In the present study, the low-dose treatment with losartan plus simvastatin did not alter SBP, DBP, LVSP, LVEDP, myocardial blood flow, left ventricular weight, and infarct size (%), but increased peak positive LV $dP/dt$. Therefore, the improvement in left
ventricular systolic function by losartan plus simvastatin might be independent of preload and afterload reduction, infarct size reduction, or inhibition of left ventricular remodeling, although peak positive LV dP/dt depends somewhat on preload and afterload. In our rat myocardial infarction model, 3 mg/kg/day losartan plus 2 mg/kg/day simvastatin but not the same dose of losartan or simvastatin alone improved left ventricular function. The result suggests that the doses of each agent might not solely influence hemodynamics but that the combination of ARBs and statins might have synergistic cardioprotective effects in myocardial ischemia, even at low-doses for both agents. Since statins have been shown to attenuate angiotensin II-induced myocyte hypertrophy in cultured neonatal rat cardiomyocytes in a manner that is probably mediated by the attenuation of oxidative stress [6], we can envision from our study that the pleiotropic, antioxidative effect of statins may potentiate the cardioprotective action of ARBs in the ischemic failing heart.

Study limitations

This study has several potential limitations. First this study did not include sham operated control data. In this study, myocardial infarction induction was performed through the left anterior descending artery ligation without following reperfusion, because we intended to exclude the impact of reperfusion injury. In most clinical settings, however, patients with acute myocardial infarction receive emergent reperfusion therapy with coronary angioplasty. So we might have to evaluate the pharmacological effects of statins plus ARBs on left ventricular function, using ischemia/reperfusion models. In addition, we did not measure serum lipid levels. To establish that a pleiotropic mechanism mediates the cardioprotective effects of statins when given in combination with ARBs, we should confirm that the cardioprotective effects are independent of lipid-lowering effects. In this study, we used peak positive LV dP/dt as a marker of left ventricular systolic function, but did not obtain data on peak negative LV dP/dt (−dP/dt), which is considered a marker of left ventricular diastolic function. Since diastolic function has recently been shown to be an important determinant of long-term prognosis in patients with chronic heart failure, the assessment of synergistic effects of ARBs and statins on diastolic function would be of interest. Finally, we did not investigate the mechanisms by which statins potentiate the effects of ARBs. The determination of the molecular basis of the cardioprotection will require additional experiments both in vivo and in vitro, especially focused on antioxidative effects.

Conclusion

The present study demonstrated that treatment with 3 mg/kg/day losartan plus 2 mg/kg/day simvastatin but not losartan or simvastatin alone improved left ventricular systolic function in a rat myocardial infarction model. The result suggests that statins given in combination with ARBs might have beneficial cardioprotective effects, even at low-doses for each agent.

References


