Influence of Angiotensin II Type 1-Receptor Antagonist CV11974 on Infarct Size and Adjacent Regional Function After Ischemia-Reperfusion in Dogs

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ABSTRACT—The presence of nonischemic regional dysfunction at the adjacent region of the ischemic myocardium was demonstrated in clinical studies. Recent studies demonstrated an angiotensin II type 1 (AT\(_1\))-receptor antagonist reduced myocardial ischemia-reperfusion injury. We investigated the role of the adjacent region after reperfusion by studying the effects of AT\(_1\)-receptor antagonist on myocardial function and infarct size. We investigated 12 open-chest anesthetized dogs undergoing 90 min of left anterior descending coronary artery occlusion followed by 4 h of reperfusion. Six dogs injected with an AT\(_1\)-receptor antagonist (CV11974) immediately after reperfusion were compared with 6 control dogs. Percent systolic shortening (%SS) was measured by two sets of the pair sonomicrometer crystals implanted to adjacent and remote nonischemic myocardium. After 4 h of reperfusion, infarct size was measured. There were no significant differences of the %SS at baseline between two regions. In both groups, %SS at adjacent region after reperfusion was significantly decreased as compared with remote region. There were no significant differences between the two groups. Infarct size, as a percentage of the area at risk, was smaller in the AT\(_1\) group than in control group (25.49 ± 7.53% vs 68.58 ± 26.88% P<0.01). AT\(_1\)-receptor antagonist reduces infarct size. This effect is not related to the change of regional myocardial function at adjacent region after reperfusion.

Keywords: AT\(_1\) antagonist, Adjacent region, Ischemia-reperfusion, Infarct size

The left ventricle undergoes marked architectural changes in response to myocardial loss by infarction. However, the mechanisms underlying left ventricular (LV) remodeling after myocardial infarction remain incompletely understood. Recent experimental and clinical studies have demonstrated the presence of nonischemic regional dysfunction at the lateral borders of the ischemic myocardium (1 – 4) and suggest that LV remodeling after myocardial infarction is associated with differences in function between noninfarcted adjacent and remote regions (5, 6).

The renin-angiotensin system is an important factor in the pathophysiology of ischemia-reperfusion injury. Angiotensin II (Ang II) has important physiologic and metabolic effects in the renin-angiotensin system. Ang II exerts important cardiac actions including positive inotropism, promotion of myocardial hypertrophy, arrhythmogenicity and coronary vasoconstriction (7). Ang II may also contribute in the short term to ischemic events by affecting hemostatic activity. Although the functional role of the Ang II remains controversial, Ang II has been shown to exert positive inotropic effects in a variety of species (8 – 10).

Ang II receptors include at least two different subtypes, type 1 receptor (AT\(_1\)) and type 2 receptor (AT\(_2\)). Both AT\(_1\) and AT\(_2\) receptors are expressed in rat heart and distributed in the myocardium. The roles of AT\(_2\) are still unknown; however, AT\(_1\) mediates the major cardiovascular effects of Ang II. Yang et al. (11) reported increased myocardial AT\(_1\)-receptor expression immediately after 25 min of ischemia and 30 min of reperfusion in the isolated buffer-perfused rat heart. Some investigators suggested that the increased AT\(_1\)-receptor expression might be related to tissue repair or to the fibrogenic response to tissue injury after ischemia (12). Ang II-receptor antagonists have been shown to pro-
tect myocardium from ischemia-reperfusion injury in experimental studies (13–15). CV11974 (2-ethoxy-1-[2′-(1H-tetrazol-5-yl)biphenyl-4-yl][methyl]-1H-benzimidazole-7-carboxylic acid), the active metabolite of active TCV-116, is an Ang II-receptor antagonist with high selectivity for the AT₁ receptor, compared with other AT₁-receptor antagonist (16).

We speculated that the effects of Ang II on regional myocardial function might be related to LV remodeling after myocardial infarction, because of the increased AT₁-receptor expression soon after ischemia. Therefore we hypothesized that Ang II may have an important role in nonischemic regional function immediately after ischemia. The purpose of this study was to investigate the short-term effects of AT₁-receptor antagonist (CV11974) on regional function at nonischemic adjacent regions after myocardial ischemia-reperfusion.

MATERIALS AND METHODS

Animal preparations

Adult mongrel 18 dogs weighing 16–21 kg were used for the study. Animals premedicated with subcutaneous ketamine (2 mg/kg) were anesthetized with intravenous sodium pentobarbital (25 mg/kg) and ventilated using a volume control respirator. Anesthesia was maintained with additional barbiturate injection (50 to 100 mg/h). The chest was opened via medium sternotomy and fourth intercostal space thoracotomy. The pericardium was opened and the heart was suspended in a pericardial cradle. Two sets of the pair sonomicrometer crystals (Triton Technology, San Diego, CA, USA) for regional length measurement were implanted to the same midwall depth of the myocardium. The crystals of each pair were positioned approximately 10 mm apart parallel to the short axis of the left ventricle. One pair at adjacent zone was implanted in an area 0–5 mm outside of the estimated position of the perfusion boundary produced by occluding the left anterior descending coronary artery (LAD). As a guide to estimate the position of the perfusion boundary, we used the epicardial vascular anatomy. Another pair at the normal zone was implanted in the posterolateral wall of the left ventricle perfused by the left circumflex coronary artery. A high-fidelity micromanometer-tipped catheter (ANP-531; Sentron, Zoetermeer, Netherlands) was placed into the carotid artery and retrogradely advanced across the aortic valve into the left ventricle (Fig. 1). A 2.0 silk suture was passed around a proximal site of the LAD, and the ends of silk were threaded through a small vinyl tube to form a snare. The coronary was occluded by pulling the snare, which was then fixed by clamping the tube with a mosquito hemostat. Myocardial ischemia was confirmed by regional cyanosis. Reperfusion was effected by releasing the snare and was confirmed by visible hyperemia over the surface.

Experimental protocols

All experiments were performed in accordance with the guidelines of Kansai Medical University Animal Research Committee. In each dog, the proximal left anterior descending coronary was occluded for 90 min and followed by 4 h of reperfusion. Segment length and hemodynamic indices were measured at baseline and 60 min after reperfusion. Hemodynamic variables were measured while the respirator was turned off at the end-expiratory state to avoid the effect of positive-pressure breathing. After control measurements recorded in the resting state, each animal was assigned to either of two groups: AT₁ group dogs were treated with AT₁-receptor antagonist after transient left coronary occlusion and control group dogs were without injection of AT₁-receptor antagonist. In the AT₁ group, the AT₁-receptor antagonist was given as an intravenous bolus (1 mg/kg) after transient left coronary occlusion and then was fol-
lowed by 60-min continuous infusion (0.1 mg/kg per min). Segment length and hemodynamic indices were also measured before and 60 min after injection of AT₁-receptor antagonist. Thereafter, the heart was excited after 4 h reperfusion.

Assessment of infarct size

In postmortem examination, the myocardial area at risk was determined by injecting 30 ml of 2% Evans Blue into the left atrium after reocclusion of the LAD just before killing the animal. After excision of the heart, both atria and the right ventricle were removed. Thereafter, the left ventricle was cut perpendicularly to the apical-basal axis in 8-mm-thick slices. The slices were incubated in 2% triphenyltetrazolium chloride for 15 min at 37°C. Triphenyltetrazolium chloride stains viable myocardium red, whereas unstained areas represent necrosis. The area at risk and the extent of necrosis were measured by planimetry using the National Institutes of Health program NIH Image 1.61 (17). The fractions of both area at risk to total slice size and infarct size to total slice size were calculated and multiplied by the weight of the slice to determine area at risk and infarct weight per slice. The planimetry was performed without the knowledge of group allocation.

Data analyses

LV end-diastolic pressure (EDP), end-systolic pressure (ESP), regional segment lengths at the adjacent and remote region, peak positive first derivative of left ventricular pressure (dP/dt_max) derived from the LV pressure signals, and electrocardiogram (ECG) were recorded on a multichannel recorder (SuperScope II; GW Instruments, Somerville, MA, USA). Time of end-systole was defined as 20 ms before the peak negative dP/dt. Segment length was normalized so that the end-diastolic length (EDL) was 10 mm. The extent of regional systolic shortening (%SS) was calculated as (EDL − end-systolic length (ESL)) / (EDL) × 100 (%).

Drugs

CV11974 was generously supplied by Takeda Chemical Industries, Ltd. (Osaka). CV11974 was dissolved in 5% Na₂CO₃ solution and then diluted with saline to the appropriate concentration. CV11974 is a highly potent and selective AT₁-receptor antagonist (16).

Statistical analyses

All values are reported as the mean ± S.D. To assess the statistical significance of changes from baseline to reperfusion in the regional length, %SS and hemodynamic indices between two groups, repeated measured ANOVA was used (SuperANOVA version 1.1; Abacus Concepts, Berkeley, CA, USA). Infarct sizes from both groups were compared by an unpaired t-test. Results with P<0.05 were considered statistically significant.

RESULTS

A total of 18 dogs were initially entered into the study. One dog in the control group died because of fatal ventricular fibrillation during the reperfusion period. Two dogs in the control group were excluded because they did not recover after two attempts to defibrillate with DC counter shock. Three dogs were also excluded because of lack of epicardial cyanosis and dyskinesis or disposition of crystals in the adjacent zone after LAD occlusion (two in the control and one in AT₁ group). Thus 12 dogs completed the entire protocol.

Systemic hemodynamics

Table 1 represents mean values for systemic hemodynamics (mean heart rate, EDP, ESP and dP/dt_max) at baseline and 60 min after reperfusion. There were no significant differences in hemodynamic indices between two groups at baseline. After reperfusion, ESP and dP/dt_max did not change significantly in either of the groups. Heart rate decreased significantly after reperfusion in both groups. These changes were not significantly different between the two groups. The changes of EDP were significantly different between the two groups by the use of ANOVA. Although EDP increased significantly after reperfusion in the control group, EDP did not change significantly in the AT₁ group.

<table>
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<th>Control</th>
<th>AT₁</th>
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<tr>
<td> </td>
<td>baseline</td>
<td>after reperfusion</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>116 ± 25</td>
<td>92 ± 25*</td>
</tr>
<tr>
<td>EDP (mmHg)</td>
<td>8.5 ± 6.5</td>
<td>13.6 ± 6.6*</td>
</tr>
<tr>
<td>ESP (mmHg)</td>
<td>101 ± 7</td>
<td>100 ± 14</td>
</tr>
<tr>
<td>dP/dt_max (mmHg/s)</td>
<td>2817 ± 386</td>
<td>2187 ± 470*</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D. *P<0.01 vs baseline. HR = heart rate, EDP = end-diastolic pressure, ESP = end-systolic pressure, dP/dt_max = maximum first derivative of left ventricular pressure.
Infarct Size and AT₁ Antagonist

Regional function
The %SS in the normal zone in the control group at the baseline level, expressed as a percent of the EDL, was not significantly different from that in the AT₁ group. There was no significant difference between the %SS in the adjacent zone in the control group and the AT₁ group. At the baseline level, regional function was not significantly different between the control and AT₁ group (Table 2). The %SS in the adjacent zone decreased significantly in both groups at 60 min after reperfusion. The %SS in the normal zone did not change significantly either of the groups.

Myocardial infarct size
The area at risk as a percentage of the left ventricle proved to be similar in the control and AT₁ group (13.60 ± 8.93% vs 17.88 ± 5.07%), as shown in Fig. 2. Infarct size, as a percentage of the area at risk, was significantly reduced in the AT₁ group (25.49 ± 7.53%) compared with the control group (68.58 ± 26.88%) (P<0.01).

DISCUSSION

Effects of AT₁-receptor antagonist on LV function
The AT₁-receptor antagonist did not change ESP significantly, whereas EDP was decreased in the AT₁ group. Previous studies suggested that the mechanism whereby the AT₁-receptor antagonist decreases EDP is venodilation (18). It was recently shown that a specific antagonist of angiotensin II receptor could decrease LV volumes and increase ejection fraction after myocardial infarction in the rat (19). However, mean arterial pressure was also reduced in that study, therefore, the improvement in LV function may have been due to a decrease in LV afterload. In the present study, regional function in the normal zone in both groups was not changed, so the AT₁-receptor antagonist showed no significant effect on ESP. In this setting, the AT₁-receptor antagonist without any changes of afterload did not affect regional function also in the adjacent zone, but reduced infarct size significantly. These data indicated that the dose of AT₁-receptor antagonist, without changing LV systolic function, had a significant beneficial effect on ischemic myocardium. In recent studies, candesartan that did not produce any hemodynamic effect clearly reduced the infarct size and improved myocardial functional recovery (15). One of the limitations of the present study is that it is possible that the results of this study were influenced by the dose used, because previous studies reported a dose-dependent effect of candesartan (15). We attempted to use a dose that was effective but did not induce hypotension. In this study, the AT₁-receptor antagonist after ischemia-reperfusion reduced infarct size at a dose that was not associated with any changes in hemodynamics and myocardial regional function in the adjacent zone. In this setting, we could not find any influences of inotropic effects of Ang II on the nonischemic regional function. Although we hypothesized that AT₁-receptor antagonist has an important role in the non-infarcted adjacent region after ischemia-reperfusion, our results indicate that there is no difference in regional function in the non-infarcted adjacent regions between the control and AT₁ group. However, infarct size was significantly reduced in the AT₁ group compared with the control group. Therefore, we suggested that the beneficial effect of AT₁ antagonist can be achieved without any influence on regional function and hemodynamics after ischemia-reperfusion.

Effect of AT₁-receptor antagonist on infarct size
This reduction in infarct size induced by the AT₁-receptor antagonist was not associated with any changes in hemodynamic parameters without EDP. Our in vivo data are consistent with recent studies in which the AT₁-receptor antagonists reduced the infarct size after myocardial is-

Table 2. Regional function

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>AT₁</th>
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<tr>
<td></td>
<td>adjacent zone</td>
<td>normal zone</td>
</tr>
<tr>
<td>Baseline</td>
<td>19.01 ± 3.44</td>
<td>20.51 ± 4.96</td>
</tr>
<tr>
<td>After reperfusion</td>
<td>12.42 ± 6.28*</td>
<td>19.09 ± 2.76</td>
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Values are expressed as the mean ± S.D. *P<0.05 vs baseline. %SS = percent systolic shortening.

Fig. 2. Area at risk (AR) as a percentage of the left ventricle (LV) and infarct size as a percentage of the AR in the control group and in the AT₁ group. *P<0.01 compared with the control.
Chemistry and reperfusion (15, 20). However, it is in contrast with some other studies performed in rabbits and dogs, in which no significant effect on infarct size was observed with losartan (21) and its active metabolite EXP 3174 (22). The reason for this discrepancy might be related to several factors such as differences in animal species, different binding characteristics of the compounds and various time and routes of drug administration. In recent studies in the canine model, the intravenously administration of CV11974 was reported to reduce infarct size after ischemia-reperfusion (23, 24). Cardiovascular effects of Ang II have been mainly attributed to AT₁-receptor activation (19, 20). The circulating and the local cardiac renin-angiotensin systems are both activated during ischemia-reperfusion, thereby enhancing the production of Ang II (7). Binding of Ang II to AT₁ receptors activates phospholipase C, increasing the susceptibility of the myocardium to ischemia-reperfusion insult (14, 25). Blocking of this process may favorably influence myocardial resistance to reperfusion damage. Furthermore, Ang II is an important regulator of norepinephrine release from sympathetic activity (7, 25). In addition, the AT₁-receptor antagonist causes an increase in Ang II level (25), which subsequently enhances formation of angiotensin and/or activates the AT₂ receptor in endothelial cells, resulting in enhanced synthesis and release of vasodilator prostaglandins, nitric oxide and/or bradykinin (26, 27). This may also have contributed to the cardioprotective effects of CV11974.

Limitations of the present study

One of the limitations of the present study might be the amount of administration of CV11974. CV11974 was used only at a single dose (25 to 34 mg total dose). In patients with hypertension, the prodrug candesartan cilexetil is only at a single dose (25 to 34 mg total dose). In patients under treatment with AT₁-receptor antagonist after acute myocardial infarction are likely to have an improved prognosis.

In conclusion, the AT₁-receptor antagonist protects the myocardium from ischemia-reperfusion injury at a dose in which it is not associated with any changes in hemodynamics and myocardial regional function. This supports the involvement of cardiac Ang II in the development of myocardial ischemia-reperfusion injury and indicates the therapeutic value of an AT₁-receptor antagonist in the setting of myocardial infarction.

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