Attenuation of heart failure due to coronary stenosis by ACE inhibitor and angiotensin receptor blocker

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Sato, Hidetoshi, Hiroyuki Yaoita, Kazuhira Maehara, and Yukio Maruyama. Attenuation of heart failure due to coronary stenosis by ACE inhibitor and angiotensin receptor blocker. Am J Physiol Heart Circ Physiol 285: H359–H368, 2003. First published March 20, 2003; 10.1152/ajpheart.00615.2002.—It is not known how the angiotensin-converting enzyme (ACE) inhibitor and angiotensin II receptor blocker (ARB) attenuate heart failure (HF) in viable ischemic hearts. To assess HF in a rat coronary stenosis (CS) model, we administered vehicle or quinapril or candesartan (or both) orally for 12 wk. Compared with the sham group, the vehicle group showed impaired myocardial perfusion, impaired coronary endothelial nitric oxide (NO) function in vitro, exhausted myocardial mitochondrial respiration, larger left ventricular (LV) dimensions and lower ejection fraction, lower LV rate of pressure development over time (dP/dt), lower slopes of LV end-systolic pressure-dimension relations (ESPDRs), and increased myocardial fibrosis. Treatment with quinapril or candesartan ameliorated these parameters without modifying the epicardial CS severity. Moreover, their combination maintained similar myocardial perfusion, despite a trend toward lower blood pressure, and showed distinctive neurohumoral modulation, normalized mitochondrial respiration, and increased ESPDR slopes. Thus improved myocardial blood flow and coronary flow reserve by quinapril or candesartan are the key to alleviate CS-induced HF, and their combination may have a therapeutic significance partly through ameliorated mitochondrial respiration and improved LV systolic function.

Because slow progressive damage of severely ischemic but viable myocardium occurs in patients with coronary artery disease (3), new approaches to prevent the worsening of ischemic heart failure (HF) are needed. Angiotensin-converting enzyme (ACE) inhibitor and angiotensin II type 1 receptor blocker (ARB) attenuate left ventricular (LV) remodeling after myocardial infarction (MI) (18), and these agents have been used to treat patients with HF. In a subpopulation of such patients, 48–66% had MI and 55–71% had angina as baseline characteristics (15, 21, 22). As shown previously (28), some drugs may have greater effects on viable areas than on nonviable areas in the ischemic heart, and it is possible that the therapeutic effects of cardiovascular agents may differ between infarcted and ischemic but viable hearts. Therefore, the antiremodeling effects of these pharmaceuticals on HF due to ischemia but not to MI need to be investigated.

The Randomized Evaluation of Strategies for Left Ventricular Dysfunction (RESOLVD) study (8) assessed the therapeutic effects of an ACE inhibitor and an ARB, alone or in combination, on HF, in which 71% of the patients had ischemic heart disease. The study revealed that there is an attenuation of the increase in LV volumes in HF patients who received both an ACE inhibitor and an ARB. Although it was assumed that reduced preload and afterload contributed in part to the antiremodeling effect of the therapies, especially in patients who received the combination therapy, it is unknown whether or not the antiremodeling effect of the combination therapy is modifying the underlying causes of HF.

The combination of an ACE inhibitor and an ARB attenuated cardiac remodeling after experimental MI (29), but there is no direct evidence that the combination therapy is beneficial in ischemic but viable hearts with coronary stenosis (CS). In the present study, we aimed to assess whether these pharmaceuticals, individually and in combination, prevent ischemic LV dysfunction and remodeling induced by CS. To examine the mechanisms of the therapeutic effects, other than on load changes, the effects of the therapy on coronary circulation, myocardial mitochondrial O$_2$ consumption (MV$_{O2}$), and neurohumoral substances were also assessed.

METHODS

Animal models. This study using rats (n = 295) conformed to the Guideline on Animal Experiments of Fukushima Medical University, the Japanese Government Animal Protection and Management Law (No. 115) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996).

Male 10-wk-old Sprague-Dawley rats (n = 208 for creation of CS) weighing 290–310 g were anesthetized by an intraperitoneal administration of 30 mg/kg pentobarbital sodium, and monitored with the use of a limb lead II electrocardiogram. After a tracheal tube was inserted and artificial ventilation was given, a left thoracotomy was performed. The left coronary artery, 1 to 2 mm below its origin from the ascending aorta, and a stainless steel thread (275 µm in diameter)
were ligated together with a needle-attached suture (size 6.0), followed by quick thread removal (28). During the brief coronary occlusion, the ST segment was elevated and it returned to the basal level after thread removal (Fig. 1). Nine rats died of ventricular fibrillation, and five rats that had persistent ST elevation after thread removal were excluded from the study. The chest was then closed, and the animals were returned to their cages, randomized, and allowed free access to food and water until they were euthanized 10 days (n = 64), 4 wk (n = 10, blindly selected from the vehicle-treated group), or 12 wk later (Fig. 1). Permanent coronary occlusion (n = 42) as a positive control and a sham operation (n = 45) as a negative control were performed on age-matched rats. Seven rats died during the coronary occlusion surgery.

**Pharmaceutical treatments.** Pharmaceutical treatments were started within 24 h after CS. An inert vehicle (vehicle group, n = 56), 10 mg·kg⁻¹·day⁻¹ quinapril (quinapril group, n = 47), 10 mg·kg⁻¹·day⁻¹ candesartan cilexetil (candesartan group, n = 47), or 10 mg·kg⁻¹·day⁻¹ of each quinapril and candesartan (combination group, n = 44) were given every morning in a blinded fashion via a cannula inserted into the mouth (Fig. 1). The doses were based on those used in previous reports (1, 7, 10, 13, 24, 25).

**Serial assessment of LV function.** To observe LV remodeling, serial echocardiographic assessments with the use of an echocardiogram (SONOS model 110CF, Hewlett-Packard) were performed under anesthesia before, 6 h, and 10 days (only in the subgroups), and 4, 8, and 12 wk after CS or coronary occlusion. The dimensions (in mm) measured by this equipment are significant to one decimal point. LV end-diastolic (LVEDD) and end-systolic dimensions (LVESD) were measured, and the ejection fraction was calculated by the Pombo method (16).

Peak systolic blood pressure (SBP) and heart rate (HR) in the awake condition were measured by the tail cuff method before the echocardiographic study at 10 days and at 4–12 wk. Before death, the anesthetized rats received cardiac catheterization and were then divided into three subgroups for evaluation of (1) myocardial blood flow (MBF) and coronary flow reserve (CFR), (2) in vitro MV O₂ [for assessment of myocardial mitochondrial respiration and of coronary microvascular endothelium-derived nitric oxide (NO) function] and circulating neurohumoral factors (assessed at only 12 wk), (3) LV end-systolic pressure-dimension relations (ESPDs) (assessed at only 12 wk), and (4) and histopathological analysis (assessed at only 12 wk) (Fig. 1). With the exception of histopathological examinations, all measurements were performed ~6 h after the last administration of the pharmaceuticals.

**MBF and CFR.** Ten days or 12 wk after the CS or sham surgery, basal MBF (in ml·min⁻¹·g⁻¹) and CFR [maximal MBF/basal MBF (ratio)] were measured in anesthetized rats by the colored microsphere method combined with diprydiamole (10 mg·kg⁻¹·min⁻¹) infusion (28) (Fig. 1). To determine the risk area, the chest was opened, the portion in which CS had been made (ties for stenosis were the markers) was occluded, and 1% Evans blue was infused at a pressure of 100 mmHg into the left ventricle through a catheter inserted from the right carotid artery.

In *vitro* MV O₂. For in *vitro* MV O₂ measurements (Fig. 1), the rats were anesthetized and the risk area was delineated as described above. The hearts were quickly excised, and myocardial muscles taken from the endocardial side within the risk area were freed from epicardium, endocardium, and connective tissue and quickly cut into small pieces weighing ~30 mg each. The heart tissue was then bathed at 37°C in Krebs solution bubbled with 20% O₂-3% CO₂-77% N₂. After equilibration, O₂ uptake was measured polarographically with the use of a Yellow Springs Instrument apparatus consisting of a YSI model 5300 biological O₂ monitor and a Clark-type O₂ electrode (YSI 5331) according to the method of Hintze et al. (27), with minor modification. We used sodium nitroprusside (SNP) instead of S-nitroso-N-acetyl-penicillamine as a NO donor. In *vitro* MV O₂ (in nmol·min⁻¹·g⁻¹) was calculated as the rate of decrease in the concentration of O₂ in the buffer. After baseline measurements, 0.1 mM bradykinin (BK), to assess BK receptor-mediated endothelial NO production, and 0.1 mM SNP to assess submaximal effect of excessive NO, was added to the tissue bath and

**Fig. 1.** The animal model (A) and a flow diagram (B) of the study. Twelve weeks after coronary stenosis, left ventricular (LV) hypokinesia and chamber dilatation were apparent. SD, Sprague-Dawley; CS, coronary stenosis; CO, coronary occlusion; V, vehicle group; Q, quinapril group; C, candesartan group; Q+C, combination group of Q and C; CATH, cardiac catheterization; NHF, neurohumoral factors; MS, microspheres; ESPDR, end-systolic pressure-dimension relation; HISTO, histopathology; sac, sacrifice; ND, not done; and MV O₂, myocardial O₂ consumption. The numbers of animals in the MS study at 10 days were 8 each in the sham, Q, C, and Q+C groups, and 7 in the V group. In the MV O₂ study at 10 days, there were five in each group.
of systolic pressure of the left ventricle) was changed to a range into the aorta from the right carotid artery. Aortic pressure was recorded by using the point-counting method (26).

**ESPDrs.** In the ESPDR study at 12 wk after the surgery, rats were anesthetized (n = 10 each group, except n = 9 in the combination group) and a polyethylene tube was inserted into the aorta from the right carotid artery. Aortic pressure measured at a dicrotic notch (considered equal to the end-systolic pressure of the left ventricle) was changed to a range of −30–270 mmHg. Paraffin sections (5 μm thick), which included the CS portion, were stained with elastica Van Gieson (6). We measured the cross-sectional areas of coronary inner lumen by light microscopy at ×400 magnification, as reported previously (28), and CS severity was given as the ratio between stenosis lumen area and normal lumen area. We also assessed CS severity at 4 wk after CS to assess its possible changes during the experimental period.

Paraffin sections of myocardial specimens were stained with elastica Masson and hematoxylin and eosin (14) for conventional histopathology. The LV free wall was divided by light microscopy at ×400 magnification, as reported previously (28), and CS severity was given as the ratio between stenosis lumen area and normal lumen area. We also assessed CS severity at 4 wk after CS to assess its possible changes during the experimental period.

Cardiac catheterization and blood sampling. Ten days or 12 wk after the surgery, cardiac catheterization was performed on all the rats just before they were euthanized (Fig. 1). At 12 wk, circulating angiotensin II and norepinephrine (NE) were measured with the use of RIA and HPLC, respectively (Fig. 1).

**Drugs.** N^\text{G}\text{-nitro-l-arginine, BK, SNP, KCN, methoxamine, and phenolamine were obtained from Sigma. Quinapril and candesartan were obtained from Mitsubishi Welpharma and from Takeda Pharmaceuticals, respectively.**

**Statistical analysis.** Data are presented as means ± SE. Statistical analysis was performed by two-way analysis of variance. If the F test results were <0.05, Bonferroni’s post hoc test was performed. A P value <0.05 was considered significant. Among multiple comparisons, statistics are presented by comparing experimental data to the data for the sham and the vehicle group, and also by comparing the data from the quinapril and candesartan groups to the combination group to assess the effect of the combination.

**RESULTS**

**Survival.** Survival rate in each group was calculated by excluding rats who died during surgery, that is, 64 rats died at 10 days and 10 rats died at 4 wk. During the 12-wk observation period, 0 (0%), 3 (9%), 2 (6%), 3 (9%), 0 (0%), and 15 (43%) rats in the sham, vehicle, quinapril, candesartan, and combination groups and coronary occlusion group died, respectively.

CS severity. At 12 wk, CS severity (%) was similar in the five groups; 2 ± 0.1 in the sham, 65 ± 14 in the vehicle, 65 ± 12 in the quinapril, 67 ± 8 in the candesartan, and 64 ± 8 in the combination groups. These values were similar to that at 4 wk in the vehicle group (65 ± 13%), suggesting that the CS severity did not change from 4 to 12 wk after CS was created.

**MBF and CFR.** At 10 days (Table 1) and 12 wk (Table 2) after CS or sham surgery, MBF of the risk area was lower only in the vehicle group compared with the sham, whereas it was greater in the quinapril, candesartan, and combination groups compared with the vehicle group. CFR of the risk area was lower in the vehicle, quinapril, candesartan, and combination groups compared with the sham group, whereas it was greater in the quinapril, candesartan, and combination groups than in the vehicle group (Tables 1 and 2).

**Hemodynamics in awake condition.** Compared with the sham, BP by the tail cuff method tended to be low (10 days after CS: see Table 1) and was lower in the vehicle (at 4 wk), quinapril, candesartan, and combination groups (at 4 to 12 wk each) and in the coronary occlusion group (at 6 h) (Fig. 2). Compared with the vehicle group, SBP was lower in the quinapril (at 12 wk), candesartan, and combination groups (at 8 and 12 wk, each). SPP of the combination group was lower than the quinapril and candesartan groups at 8 wk. HR did not differ among the six groups at any time (Fig. 2).

**Echocardiography.** As long as 10 days after CS (Table 1), compared with the sham group, LVEDD did not change in the four CS groups, although that of the vehicle group tended to be higher, and LVEDS increased and LVEF decreased in the four CS groups. Compared with the vehicle group, LVEDS decreased and LVEF increased in the quinapril, candesartan, and combination groups.

Compared to the sham, LVEDD was greater in the vehicle group at 4 to 12 wk and in the coronary occlusion group at 6 h, and at 4 to 12 wk, and was also greater in the quinapril group at 4 wk and the candesartan group at 8 and 12 wk (Fig. 3). Compared with the vehicle group, the coronary occlusion group showed larger LVEDD from 6 h to 12 wk after surgery, but LVEDD was smaller at 8 to 12 wk in the quinapril, candesartan, and combination groups.

Compared with the sham, LVEDS was greater in the vehicle and coronary occlusion groups at 6 h, and 4 to 12 wk, and was also greater in the quinapril group at 6 h and 4 wk, the candesartan group at 6 h, and 4 to 12 wk, and the combination group at 6 h (Fig. 3). Compared with the vehicle group, LVEDS was smaller in
Table 1. Data for hemodynamics, cardiac dimensions, and in vitro MV\textsubscript{O}_{2} 10 days after coronary stenosis or sham surgery

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 13)</th>
<th>Coronary Stenosis + Vehicle (n = 12)</th>
<th>Coronary Stenosis + Quinapril (n = 13)</th>
<th>Coronary Stenosis + Candesartan (n = 13)</th>
<th>Coronary Stenosis + Quinapril + Candesartan (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure</td>
<td>5.6 ± 0.1</td>
<td>7.0 ± 0.4</td>
<td>6.6 ± 0.2</td>
<td>6.5 ± 0.2</td>
<td>6.5 ± 0.1</td>
</tr>
<tr>
<td>LV end-diastolic pressure</td>
<td>121 ± 8</td>
<td>118 ± 9</td>
<td>113 ± 8</td>
<td>111 ± 8</td>
<td>109 ± 10</td>
</tr>
<tr>
<td>LV end-diastolic dimension, mm</td>
<td>115 ± 4</td>
<td>115 ± 4</td>
<td>115 ± 4</td>
<td>116 ± 5</td>
<td>105 ± 7</td>
</tr>
<tr>
<td>LV ejection fraction, ratio</td>
<td>0.80 ± 0.01</td>
<td>0.63 ± 0.04†</td>
<td>0.71 ± 0.01§</td>
<td>0.69 ± 0.02†</td>
<td>0.73 ± 0.01§</td>
</tr>
<tr>
<td>LV systolic pressure, mmHg</td>
<td>2 ± 1</td>
<td>11 ± 3†</td>
<td>6 ± 2§</td>
<td>6 ± 2§</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mmHg</td>
<td>5 ± 2§</td>
<td>5 ± 2§</td>
<td>5 ± 2§</td>
<td>5 ± 2§</td>
<td>5 ± 2§</td>
</tr>
<tr>
<td>Heart rate by catheterization, beats/min</td>
<td>394 ± 6</td>
<td>421 ± 5§</td>
<td>407 ± 6</td>
<td>411 ± 7</td>
<td>406 ± 5</td>
</tr>
<tr>
<td>LV weight, g</td>
<td>1.1 ± 0.1</td>
<td>1.4 ± 0.04†</td>
<td>1.2 ± 0.1§</td>
<td>1.2 ± 0.1§</td>
<td>1.2 ± 0.1§</td>
</tr>
<tr>
<td>Myocardial blood flow, ml\cdot kg\textsuperscript{−1}\cdot g\textsuperscript{−1}</td>
<td>4.4 ± 0.4</td>
<td>2.1 ± 0.5†</td>
<td>3.8 ± 0.4§</td>
<td>3.7 ± 0.4§</td>
<td>3.9 ± 0.2§</td>
</tr>
<tr>
<td>Coronary flow reserve, ratio</td>
<td>2.2 ± 0.1</td>
<td>1.3 ± 0.1†</td>
<td>1.9 ± 0.1§</td>
<td>1.8 ± 0.1§</td>
<td>1.9 ± 0.1§</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals in each group. MV\textsubscript{O}_{2}, myocardial O\textsubscript{2} consumption; LV, left ventricular; \(\pm\)dP/\(\pm\)dt, rate of pressure development and relaxation over time. In group, the animals were divided into the microsphere study (n = 8 each in sham, quinapril, and candesartan, and n = 7 in vehicle) or in vitro MV\textsubscript{O}_{2} study (n = 5 per group). \(\ast\)P < 0.05, \(\dagger\)P < 0.01 vs. sham; \(\ddagger\)P < 0.05, \(\S\)P < 0.01 vs. vehicle.

Table 2. Data for hemodynamics, in vitro MV\textsubscript{O}_{2}, and neurohumoral factors 12 wk after coronary stenosis, occlusion, or sham surgery

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 32)</th>
<th>Coronary Occlusion (n = 20)</th>
<th>Coronary Stenosis + Vehicle (n = 31)</th>
<th>Coronary Stenosis + Quinapril (n = 32)</th>
<th>Coronary Stenosis + Candesartan (n = 31)</th>
<th>Coronary Stenosis + Quinapril + Candesartan (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV systolic pressure, mmHg</td>
<td>124 ± 6</td>
<td>125 ± 10</td>
<td>122 ± 5</td>
<td>113 ± 4</td>
<td>117 ± 2</td>
<td>100 ± 6</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mmHg</td>
<td>3 ± 1</td>
<td>20 ± 5§</td>
<td>11 ± 2†</td>
<td>5 ± 2§</td>
<td>6 ± 2§</td>
<td>2 ± 1§</td>
</tr>
<tr>
<td>+LVDp/dt, mmHg/s</td>
<td>+8,470 ± 625</td>
<td>+4,002 ± 329†</td>
<td>+4,870 ± 266†</td>
<td>+6,662 ± 410*</td>
<td>+6,265 ± 688*</td>
<td>+7,980 ± 471§</td>
</tr>
<tr>
<td>−LVDp/dt, mmHg/s</td>
<td>−5,811 ± 432</td>
<td>−2,610 ± 102†</td>
<td>−3,250 ± 202†</td>
<td>−4,526 ± 265*</td>
<td>−4,243 ± 495†</td>
<td>−5,033 ± 377§</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>399 ± 11</td>
<td>418 ± 9</td>
<td>432 ± 9§</td>
<td>421 ± 10</td>
<td>400 ± 9</td>
<td>406 ± 11</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.3 ± 0.02</td>
<td>2.7 ± 0.5§§</td>
<td>1.6 ± 0.1†</td>
<td>1.3 ± 0.1§</td>
<td>1.4 ± 0.1§</td>
<td>1.2 ± 0.1§</td>
</tr>
<tr>
<td>Myocardial blood flow, ml\cdot min\textsuperscript{−1}\cdot g\textsuperscript{−1}</td>
<td>4.4 ± 0.5</td>
<td>ND</td>
<td>2.1 ± 0.4†</td>
<td>3.8 ± 0.6‡</td>
<td>3.6 ± 0.3§</td>
<td>3.7 ± 0.4‡</td>
</tr>
<tr>
<td>Coronary flow reserve, ratio</td>
<td>2.2 ± 0.1</td>
<td>ND</td>
<td>1.3 ± 0.1†</td>
<td>1.9 ± 0.1§</td>
<td>1.8 ± 0.1§</td>
<td>1.8 ± 0.1§</td>
</tr>
<tr>
<td>In vitro MV\textsubscript{O}_{2}, nmol\cdot min\textsuperscript{−1}\cdot g\textsuperscript{−1}</td>
<td>156 ± 12</td>
<td>ND</td>
<td>396 ± 33‡</td>
<td>282 ± 33‡</td>
<td>288 ± 33‡</td>
<td>189 ± 12‡</td>
</tr>
<tr>
<td>Plasma norepinephrine, pmol/ml</td>
<td>1.2 ± 0.2</td>
<td>2.5 ± 0.8§</td>
<td>2.3 ± 0.3†</td>
<td>1.5 ± 0.2‡</td>
<td>1.8 ± 0.2</td>
<td>1.2 ± 0.3§</td>
</tr>
<tr>
<td>Plasma angiotensin II, nmol/l</td>
<td>273 ± 76</td>
<td>440 ± 238</td>
<td>328 ± 57</td>
<td>106 ± 30*</td>
<td>504 ± 108†</td>
<td>118 ± 29§</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals in each group. ND, not done. Heart weight, plasma, norepinephrine, angiotensin II, and in vitro MV\textsubscript{O}_{2} were measured in 10 animals from each group. The microsphere study was performed in 11 or 12 animals from each group. \(\ast\)P < 0.05, \(\dagger\)P < 0.01 vs. sham; \(\ddagger\)P < 0.05, \(\S\)P < 0.01 vs. vehicle.
the quinapril and candesartan groups at 8 and 12 wk and in the combination group at 4 to 12 wk (also $P < 0.05$ combination group vs. the candesartan-group at 8 and 12 wk).

Compared with the sham group, LVEF was lower in the all groups at 6 h and also in the vehicle group and the coronary occlusion group at 4 to 12 wk (Fig. 3). Compared with the vehicle group, LVEF was lower in the coronary occlusion group but was higher at 4 to 12 wk in the quinapril, candesartan, and combination groups. LVEF in the combination group was higher than that in the candesartan group at 8 and 12 wk.

ESPDRs. In some cases, end-systolic pressure was not largely elevated by methoxamine infusion, especially in the vehicle group and coronary occlusion group, probably due to LV dysfunction (Fig. 4). Com-

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Fig. 2. Changes in peak systolic blood pressure (A) and heart rate (B) in conscious animals measured by the tail-cuff method. $n$, Initial number of animals, except those euthanized 10 days after coronary stenosis or sham surgery. w, weeks; h, hours.

Fig. 3. Changes in echocardiographic parameters [LV end-diastolic (LVEDD; A) and end-systolic dimensions (LVESD; B), and ejection fraction (LVEF)]. $n$, Initial number of animals, except those euthanized 10 days after coronary stenosis or sham surgery; C).
pared with the sham group (36 ± 2), the slopes (in mmHg/mm) of ESPDRs were lower in the coronary occlusion group (11 ± 1) and the vehicle group (13 ± 1), and were also lower in the quinapril (24 ± 1), candesartan (22 ± 2; P < 0.01 each), and combination (29 ± 2; P < 0.05) groups. Compared with the vehicle group, the slopes were higher (P < 0.01) in the quinapril, candesartan, and combination groups, and compared with the quinapril and candesartan groups, the slopes were much higher in the combination group (P < 0.05, each). The predicted mean values (in mm) extrapolated to the x-axis at zero pressure in the vehicle (−2.5 ± 0.5), quinapril (−0.1 ± 0.3), candesartan (−1.9 ± 0.7), and combination (−0.4 ± 0.2) groups and in the coronary occlusion group (−2.9 ± 1.1) were not different from the sham (−1.1 ± 0.3).

**Hemodynamics in anesthetized condition.** Ten days after CS, compared with the sham, LVEDP increased, and ± or +LV rate of pressure development over time (dP/dt) decreased in the vehicle, quinapril, and candesartan groups (Table 1). Compared with the vehicle group, LVEDP decreased in the quinapril, candesartan, and combination groups, and +LVdP/dt increased in the combination group.

Twelve weeks after surgery (Table 2), there was some discrepancy between the LV peak systolic pressure measured by cardiac catheterization and by the tail-cuff method (Fig. 2), probably due in part to

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Fig. 4. LV ESPDRs in the sham (A) and coronary stenosis groups treated with vehicle (B), quinapril (C), candesartan (D), quinapril and candesartan (E), and the coronary occlusion (F) group at 12 wk. Different symbols represent individual animals. Solid lines indicate linear regressions. SBP, systolic blood pressure.
whether or not the animals were anesthetized. Compared with the sham group, LVEDP increased in the vehicle group and the coronary occlusion group. Compared with the vehicle group, LVEDP decreased in the quinapril, candesartan, and combination groups. Compared with the sham group, LVDp/dt was lower in the vehicle group and the coronary occlusion group, and was also lower in the quinapril, candesartan, and combination groups, except for +LVDp/dt in the combination group. Heart weight was greater in the vehicle group and the coronary occlusion group compared with the sham, and less in the quinapril, candesartan, and combination groups than in the vehicle group. Circulating NE was higher in the vehicle group and the coronary occlusion group compared with the sham, and lower in the quinapril and combination groups compared with the vehicle group. Circulating angiotensin II was higher in the candesartan group than in the sham and lower in the quinapril and combination groups than in the vehicle group.

In vitro $\dot{MV}O_2$. Both 10 days (Table 1) and 12 wk (Table 2) after CS, compared with the sham, the values for in vitro $\dot{MV}O_2$ (in nmol·min$^{-1}$·wet wt g$^{-1}$) were higher in the vehicle, quinapril, and candesartan groups but not in the combination group, and lower in the quinapril, candesartan, and combination groups than in the vehicle group at the basal state. Both 10 days (Fig. 5A) and 12 wk (Fig. 5B) after surgery in the sham, quinapril, and combination groups, in vitro $\dot{MV}O_2$ decreased after BK to a level nearly comparable to that of SNP. In the vehicle group, the decrease in the in vitro $\dot{MV}O_2$ after BK was smaller than in the other groups, resulting in a lower value of the index than in the other groups.

Histopathology. In the vehicle group, the myocardial interstitial area within the risk area was 19.1 ± 4.9%. Compared with the vehicle group, the areas of fibrosis were larger ($P < 0.01$) in the coronary occlusion group (95.8 ± 0.2%) but were lower ($P < 0.05$, each) in the sham (5.3 ± 0.5%), quinapril (8.2 ± 2.0%), and candesartan (8.2 ± 3.2%), and combination (4.6 ± 1.5%) groups (no significance among these four groups).

DISCUSSION

In the present study we investigated the effects of an ACE inhibitor, an ARB, and their combination on the development of ischemic LV dysfunction and remodeling induced by CS, and our findings can be summarized as follows. First, reduction of MBF and CFR by CS evoked LV dysfunction and LV remodeling in rats. Quinapril and candesartan attenuated the reduction of MBF and CFR without modifying the CS severity, induced reduction of myocardial fibrosis, and attenuated the development of LV dysfunction and remodeling. Second, compared with each agent alone, the combination of quinapril and candesartan did not cause a conspicuous change in myocardial perfusion (although there was a trend of reduced perfusion pressure) in the resting condition, but the combination of them normalized in vitro $\dot{MV}O_2$ and also improved ESPDRs more than either of them alone.

In vitro $\dot{MV}O_2$. Persistent stenosis of the coronary artery leads to cardiac dysfunction in animals as well as in human. In 1992, Anversa et al. (2) reported on LV dysfunction in the CS rat model, followed by our study 2002 (28). In the present study, reduction of MBF and CFR and impairment of coronary NO function in vitro in the risk area caused by CS preceded LV remodeling, such as the increases in LVEDD. In vitro $\dot{MV}O_2$ was greater in the coronary stenosed rats than in the sham. Previously, Xie et al. (27) showed that in vitro $\dot{MV}O_2$ in the nonbeating state was mainly due to mitochondrial respiration and that this $\dot{MV}O_2$ was suppressed in normal tissue by NO donors and by BK through NO-mediated suppression of the activities of mitochondrial enzymes and its interaction with the heme group of cytochrome c oxidase. Therefore, this $\dot{MV}O_2$ does not reflect that in the beating heart. Mitochondrial function, which has a pivotal role in producing ATP, is

![Fig. 5. Functional activities (%) of coronary endothelial NO in vitro in risk area myocardium | changes in the in vitro $\dot{MV}O_2$ caused by BK 0.1 mM administration/maximal changes in the in vitro $\dot{MV}O_2$ caused by administration of 0.1 mM sodium nitroprusside (SNP) 10 days (A; n = 5 each group) and 12 wk after coronary stenosis or sham operation (B; n = 10 each group). In the sham, quinapril, candesartan, and combination groups, BK decreased in vitro $\dot{MV}O_2$ to nearly the maximum changes caused by SNP. However, in the vehicle group, the decrease in the in vitro $\dot{MV}O_2$ caused by BK was smaller than that caused by SNP, thus resulting in a smaller value of the index than in the other groups.](http://ajpheart.physiology.org/)

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required to keep cardiac viability and contractility. The increase in in vitro MVo$_2$ may mean exhaustion of mitochondrial respiration, which is not efficient for producing ATP. The greatest decrease of inefficiently increased in vitro MVo$_2$ by the combination therapy suggests amelioration of abnormal mitochondrial respiration and improvement of the efficiency of ATP production (27).  

**Mechanisms of improved myocardial perfusion and attenuation of HF by treatments.** There are several possibilities for the mechanisms involved in the improved myocardial perfusion caused by the treatments. First, in the presence of CS, there is interplay between the hemodynamics of the stenosis and microcirculatory resistance (19). NO modulates coronary microcirculation and plays a pivotal role in modulating MBF (12). In the present study, treatments with quinapril, candesartan, and their combination increased MBF and CFR without modifying the CS severity suggesting their effect to the nonepicardial coronary vessels. Xie et al. (27) assumed that the decrease in in vitro MVo$_2$ by BK reflects NO production in coronary small vessels in the myocardial samples taken from the endocardial side. In the present study, we did not assess whether such response of in vitro MVo$_2$ by BK reflects NO production in vivo and whether the cardiac sources of such NO production by BK are coronary small vessels. Thus although the mechanism of the increases in MBF and CFR in the treatment groups is still unclear, there is a possibility that the treatments improved myocardial perfusion via NO production. This possibility needs to be confirmed in the future study. Second, MBF and CFR are affected by the diastolic time fraction, although we did not assess it in the present study. In this regard, the ACE inhibitor has been shown to decrease the QT interval corrected by the R-R interval (17), leading to the increase in the diastolic time fraction and resultant increase in the flow (11). Third, remodeling of the microcirculation in the ischemic area may be induced with the use of an ACE inhibitor and an ARB, especially in combination, and this may lead to an alleviation of microcirculatory disturbance. Fourth, we did not measure collateral circulation in rats because of the technical limitation of measuring it in small animals. Thus the possibility that the improvement of myocardial perfusion in the risk area resulted from the development of collateral circulation by the treatments, especially in the ACE inhibitor and combination groups as reported previously (23) cannot be ruled out in this study.  

The effect of the combination of an ACE inhibitor and an ARB on cardiac contraction has been reported in pacing-induced HF (20). In our ischemic HF model, the combination of the two pharmaceuticals, but not their use individually, normalized MVo$_2$ and also improved the ESPDRs, indicating preservation of contractile performance. In the RESOLVD study (8), candesartan, enalapril, and their combination showed no significant differences in outcome with respect to exercise capacity and mortality in HF. However, the combination seemed more beneficial than either agent alone for preventing LV remodeling. Although it was not shown how the beneficial effects of the combination were modified by the underlying causes of HF in the RESOLVD study, our results provide new information, which suggests that improvement of coronary microcirculation, normalization of abnormally exhausted mitochondrial MVo$_2$ and improvement of LV systolic function, may contribute to the effects of the combination therapy in CS-induced HF.  

**Effect of load reduction on cardiac dysfunction and remodeling.** There was a reduction of SBP in the quinapril, candesartan, and combination groups compared with the vehicle group (Fig. 2). In addition, at 8 wk, SBP of the combination group was lower than in the other two. From these data, it may be speculated that the SBP-lowering effect may have contributed to increases in LVEF caused by either monotherapy or the combination therapy. In our previous study (28) that used the same CS model, the a-blocker bunazosin lowered SBP, whereas the increased LVEDP, lowered ±LVdA/dt, and reduced MBF and CFR were not ameliorated. Accordingly, load reduction not in association with increases in MBF and CFR may not necessarily play a key role in the amelioration of a subtype of HF, which is caused by severe ischemia with ~50% MBF reduction. We assume that in the combination group, restored MBF and CFR despite lowered blood pressure may have contributed to a favorable myocardial O2 demand-supply relation leading to myocardial protection.  

**Neurohumoral modulation.** Differences in neurohumoral modulation may have partly affected the results of treatment in each group. Namely, at 12 wk with the doses utilized, the combination therapy decreased LVESD and increased LVEF compared with therapy with an ARB alone but did not significantly compared with therapy with an ACE inhibitor alone. An ACE inhibitor attenuated increases in circulating NE and angiotensin II comparable to the results of the combination therapy. In contrast, an ARB induced a significant anti-LV remodeling effect, but increased the level of angiotensin II. Consequently, the beneficial action on coronary circulation and ventricular remodeling mediated by angiotensin II type 2 receptors may be working in association with type 1 receptor blockade in the candesartan group. Because the cardiac effects of the ACE inhibitor depend on BK-mediated NO synthesis in addition to blockade of angiotensin II signals, it has been postulated that the combination of an ACE inhibitor and an ARB causes activation of the NO pathway and angiotensin II receptor blockade without an increase in the level of angiotensin II. Actually, this mechanism was operating in pacing-induced HF (20) and our results suggest that this may also be the case in failing hearts with ischemic but viable myocardium.  

In the Dahl salt-sensitive HF model, cardiac endothelin-1 production is involved in the development of LV dysfunction (9), and the combination of an ACE inhibitor and an ARB ameliorated diastolic dysfunction and improved survivals (5). Further study is needed to assess whether cardiac endothelin-1 is also
involved in the pathogenesis of CS-induced LV dysfunction and remodeling, including the possibility of it being modified by the treatments.

Species differences. Although we would like to extrapolate the results of this study to human therapy, we must consider the relevant differences between rats and human. In human, chymase is a predominant enzyme in converting angiotensin I to angiotensin II, and thus it may be beneficial to add the ARB to the ACE inhibitor. In contrast, rats lack chymase activity. Therefore, it is plausible that the blocking of angiotensin II-mediated effects by ACE inhibition may be more efficacious in our model than in humans.

Dosing of pharmaceuticals. We do not have data as to whether the increased dose, such as 20 mg·kg⁻¹·day⁻¹, of quinapril may have a greater effect on our HF model and whether the addition of candesartan to the increased dose of quinapril may have a further effect. However, it is probable that in our rat model lacking chymase activity, when a much higher dose of ACE inhibitor is administered, the angiotensin II-mediated signals may be almost completely blocked, and there would be no additive effect of ARB. Therefore, the result that the combination of quinapril and candesartan ameliorated mitochondrial respiration and ESPDRs more than either agent alone might be in part because the ARB was added to a submaximal (but not maximal) dose of ACE inhibitor.

Study limitations. There are several limitations to this study. First, we assessed antiremodeling effects with only a single dose of each agent. Dose-response relationships need to be investigated to determine the maximal effects of each agent alone and in combination. Second, the effect of the agents on survival rates is one of the most important issues from a therapeutic point of view. To assess this in our model, much longer periods of observation of the animals would be required. Third, we assessed neurohumoral modulation by the treatments only in the circulation but not at the tissue level. Fourth, the molecular mechanisms of the effect of the combination of the ACE inhibitor and the ARB on LV pump function and MVO₂ in our model need to be determined.

In summary, oral administration of quinapril and candesartan improved myocardial perfusion and preserved abnormal changes in the LV structure and function, myocardial fibrosis, MVO₂, and neurohumoral factors, which were induced by CS. The combination of the two agents augmented blood pressure lowering compared with each agent alone, and their combination improved LV contractile performance irrespective of loading condition and also normalized exhausted MVO₂ in vitro in CS-induced HF.

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