Effects of Combination of ACE Inhibitor and Angiotensin Receptor Blocker on Cardiac Remodeling, Cardiac Function, and Survival in Rat Heart Failure

Shokei Kim, MD, PhD; Minoru Yoshiyama, MD; Yasukatsu Izumi, MD; Hitomi Kawano, MD; Manabu Kimoto, MD; Yumei Zhan, PhD; Hiroshi Iwao, MD

Background—The mechanism and treatment of diastolic heart failure are poorly understood. We compared the effects of an ACE inhibitor, an angiotensin receptor blocker (ARB), and their combination on diastolic heart failure in Dahl salt-sensitive (DS) rats.

Methods and Results—DS rats fed an 8% NaCl diet from 7 weeks of age were treated with benazepril 10 mg/kg alone, valsartan 30 mg/kg alone, or combined benazepril and valsartan at 5 and 15 mg/kg, respectively, or at 1 and 3 mg/kg, respectively. At 16 weeks of age, DS rats exhibited prominent concentric left ventricular (LV) hypertrophy and diastolic dysfunction with preserved systolic function, as estimated by echocardiography. Despite comparable hypotensive effects among all drug treatments, the combination of benazepril 5 mg/kg and valsartan 15 mg/kg improved diastolic dysfunction and survival in DS rats more effectively than ACE inhibitor or ARB alone. Furthermore, the increase in LV endothelin-1 levels and hydroxyproline contents in DS rats was significantly suppressed only by combined benazepril and valsartan, and LV atrial natriuretic peptide mRNA upregulation in DS rats was suppressed to a greater extent by the combination therapy than monotherapy.

Conclusions—The combination of ACE inhibitor and ARB, independently of the hypotensive effect, improved LV phenotypic change and increased LV endothelin-1 production and collagen accumulation, diastolic dysfunction, and survival in a rat heart failure model more effectively than either agent alone, thereby providing solid experimental evidence that the combination of these 2 agents is more beneficial than monotherapy for treatment of heart failure.

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Key Words: heart failure ■ angiotensin ■ survival

Accumulating evidence supports the notion that angiotensin AT1 receptor blockers (ARBs) have nearly the same favorable effects as ACE inhibitors on cardiac hypertrophy, remodeling, and function or heart failure.1-5 However, pharmacological profiles are substantially different between ARB and ACE inhibitors, because ARB can inhibit the action of angiotensin II (Ang II) generated through not only ACE but also the alternative pathways,6 whereas ACE inhibitors block the breakdown of bradykinin.7 Thus, combination therapy with ACE inhibitor and ARB may offer benefits beyond those of either agent alone. In fact, combined administration of ACE inhibitor and ARB is shown to decrease blood pressure in hypertensive rats8,9 and normotensive volunteers10 and improve cardiac function or hemodynamic status in pacing-induced pig heart failure11 and posts ischemic rat heart failure12 more than either intervention alone. Furthermore, several recent pilot clinical studies suggest that the combination of ACE inhibitor and ARB may be more beneficial in heart failure than either agent alone.13-15 As yet, however, the usefulness of combination therapy with these 2 agents in heart failure has not been demonstrated, and the pharmacological mechanism underlying the benefit of the combination therapy is not defined.

Despite advances in pharmacological therapy for congestive heart failure, mortality due to heart failure remains high. Diastolic heart failure in particular, which is caused by left ventricular (LV) diastolic dysfunction without systolic dysfunction and constitutes 30% to 50% of heart failure,16 has a poor prognosis.17 A specific therapeutic strategy for diastolic heart failure has not yet been established. Dahl salt-sensitive (DS) rats are shown to develop not only hypertension18 but also heart failure19,20 by a high-salt loading. A recent study showed that DS rats fed an 8% NaCl diet from 7 weeks of age develop LV diastolic dysfunction with preserved systolic function and die of congestive heart failure.20 In the present study, using this model, we compared in detail the effects of
ACE inhibitor, ARB, and a combination of the 2 on diastolic heart failure. We obtained evidence that the combination of ACE inhibitor and ARB may be a poten
tive therapeutic strategy for treatment of diastolic heart failure.

Methods

Experimental Animals and Protocol

All procedures were in accordance with institutional guidelines for animal research. DS rats (DIS/Eis, Eisai, Tokyo, Japan) were used in the present study. After weaning, DS rats were fed a 0.3% NaCl (low-salt) diet until 7 weeks of age. At 7 weeks of age, they were switched to an 8% NaCl diet. The rats were divided into 5 groups and treated with (1) vehicle (0.5% carboxymethylcellulose solution), (2) benazepril 10 mg·kg⁻¹·d⁻¹, (3) valsartan 30 mg·kg⁻¹·d⁻¹, (4) combined benazepril 5 mg·kg⁻¹·d⁻¹ and valsartan 15 mg·kg⁻¹·d⁻¹, and (5) combined benazepril 1 mg·kg⁻¹·d⁻¹ and valsartan 3 mg·kg⁻¹·d⁻¹. All drugs were periodically measured by the tail-cuff method at 4 to 5 hours after oral dosing, when these drugs exhibited the maximal hypotensive effects. At 10 and 14 weeks of age, DS rats were housed individually in metabolic cages, and 24-hour urine was collected in a flask containing 6N HCl for measurement of catecholamine concentrations. At 16 weeks of age, under ether anesthesia, arterial blood was immediately collected via the abdominal aorta, and plasma was collected by centrifugation and stored at −80°C until use. The heart was immediately excised, the LV was separated from the atria and the right ventricle, and they were immediately frozen in liquid nitrogen, and stored at −80°C until use.

Echocardiographic Study

Transthoracic echocardiographic studies were performed on 16-week-old DS rats with an echocardiographic system equipped with a 12.0-MHz phased-array transducer (SONOS 5500; Agilent Technology) as previously described in detail. In brief, rats were lightly anesthetized with intraperitoneal injection of ketamine HCl (25 to 50 mg/kg) and xylazine (5 to 10 mg/kg), M-mode tracings were recorded through the anterior and posterior LV walls at the papillary muscle level to measure LV end-diastolic dimension, fractional shortening, LV anterior wall thickness at end diastole, and posterior wall thickness at end diastole. To calculate LV end-diastolic volume and LV ejection fraction, end-diastolic and end-systolic areas were obtained from the 4-chamber view, as described. Pulse-wave Doppler spectra (E and A waves) of mitral inflow were recorded from the apical 4-chamber view, with the sample volume placed near the tips of the mitral leaflets and adjusted to the position at which velocity was maximal and the flow pattern laminar.

RNA Preparation and Northern Blot Analysis

All procedures were performed as described in detail in our previous reports. In brief, 20 μg of total RNA samples from individual LVs were subjected to 1% agarose gel electrophoresis and transferred to nylon membranes, and hybridization was carried out with [³²P]dCTP-labeled cDNA probe for atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), collagen type I, GAPDH, or an oligonucleotide probe complementary to α-myosin heavy chain (MHC) (5'-TTGGTGGGATAGCAACAGCGA-3'). The densities of an individual mRNA band were measured with a bioimaging analyzer (BAS-2000, Fuji Photo Film Co).

Measurement of Cardiac Endothelin-1 Levels

LV endothelin-1 (ET-1) contents were measured by a minor modification of the method of Iwanaga et al. In brief, LV tissue was homogenized with a Polytron homogenizer in 10 volumes of 1 mol/L acetic acid containing 0.1% Triton-X, boiled for 7 minutes, and centrifuged at 20 000g for 30 minutes at 4°C. ET-1 peptide was extracted from the resulting supernatant with a Sep-Pak C18 cartridge and measured by means of a sandwich enzyme immunoassay kit (Immuino-Biological Laboratories). The sensitivity of this enzyme immunoassay kit was 0.78 pg/mL, and the cross-reactivities with ET-3 and big ET-1 were both <0.1%.

Biochemical Measurement

To estimate cardiac collagen content, tissue hydroxyproline content was determined by hydrolysis of the sample with HCl followed by high-performance liquid chromatography (HPLC). Urinary catecholamines were measured by HPLC with an automated HPLC analyzer.

Effect on Survival Rate

To examine the effect on survival, 7-week-old DS rats were fed an 8% NaCl diet and were subjected to each drug treatment, as described in Results. Animals were carefully monitored, and deaths were recorded every day. Survival rates were compared among groups at 20 weeks after the start of drug treatment.

Statistics

Results were expressed as mean±SEM. The data on blood pressure were analyzed by 2-way ANOVA, and the differences between each group at each time point were determined by the least-squares mean test. For other data, statistical significance was determined by 1-way ANOVA followed by Duncan’s multiple range test. Survival was analyzed by the standard Kaplan-Meier analysis with log-rank test and χ² analysis. In all tests, differences were considered statistically significant at a value of P<0.05.

Results

Blood Pressure and Cardiac Weight

As shown in Figure 1, DS rats fed a high-salt diet (8% NaCl) from 7 weeks of age progressively developed hypertension. All drug treatments reduced blood pressure of DS rats to only a slight extent and to a comparable degree.

As shown in Table 1, LV weight in 16-week-old DS rats fed a high-salt diet was larger than in those fed a low-salt diet (P<0.01). LV weight, corrected for body weight, of DS rats was significantly reduced by all drug treatments (P<0.01).

Figure 1. Time course of blood pressure of DS rats. DS rats were fed a high-salt diet from 7 weeks of age (arrow) and treated with vehicle (Veh, n=12), benazepril 10 mg·kg⁻¹·d⁻¹ [Ben (10), n=10], valsartan 30 mg·kg⁻¹·d⁻¹ [Val (30), n=10], benazepril 5 mg·kg⁻¹·d⁻¹ combined with valsartan 15 mg·kg⁻¹·d⁻¹ [Ben (5)+Val (15), n=10], or benazepril 1 mg·kg⁻¹·d⁻¹ combined with valsartan 3 mg·kg⁻¹·d⁻¹ [Ben (1)+Val (3), n=10]. Low Na (n=10) indicates DS rats fed a low-salt diet throughout experiments.
Cardiac Phenotype–Related Gene Expression

As shown in Figure 2, LV α-MHC mRNA levels in DS rats fed a high-salt diet were significantly decreased, and this decrease was normalized by the drug treatments, except for benazepril 10 mg/kg. LV ANP mRNA levels in DS rats fed a high-salt diet were 33-fold (P<0.01) larger than in those fed a low-salt diet. All drug treatments significantly reduced LV ANP mRNA levels in DS rats. However, the combination of benazepril 5 mg/kg and valsartan 15 mg/kg suppressed the upregulation of LV ANP to a greater extent than any other drug treatments (P<0.05). LV BNP mRNA levels in DS rats were increased by 3.2-fold by a high-salt diet, and this increase was significantly (P<0.01) and comparably inhibited by all drug treatments.

Cardiac Collagen Gene Expression and Hydroxyproline Content

As shown in Figure 3A, LV collagen type I mRNA levels were increased in DS rats fed a high-salt diet, which was significantly decreased by all drug treatments. Figure 3B indicates that LV hydroxyproline contents in DS rats fed a high-salt diet were 2.4-fold higher than those fed a low-salt diet (3.69±0.46 versus 1.56±0.12 μmol/g tissue, P<0.01). Only the combination of benazepril 5 mg/kg and valsartan 15 mg/kg significantly decreased LV hydroxyproline contents in DS rats (2.34±0.28 μmol/g tissue, P<0.05).

Cardiac ET-1 Content

Figure 4 indicates LV ET-1 levels in each group of DS rats. Sixteen-week-old DS rats fed a high-salt diet had 2.2-fold

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BW, g</th>
<th>LV, mg</th>
<th>RV, mg</th>
<th>LV/BW, mg/g</th>
<th>RV/BW, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Na</td>
<td>7</td>
<td>401±10*</td>
<td>779±19*</td>
<td>182±4</td>
<td>1.94±0.02*</td>
<td>0.454±0.013*</td>
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<td>High Na Veh</td>
<td>12</td>
<td>314±6</td>
<td>1140±26</td>
<td>186±3</td>
<td>3.65±0.08</td>
<td>0.595±0.012</td>
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<td>Ben (10)</td>
<td>9</td>
<td>327±7</td>
<td>1111±18</td>
<td>182±7</td>
<td>3.40±0.04*</td>
<td>0.557±0.018</td>
</tr>
<tr>
<td>Val (30)</td>
<td>10</td>
<td>366±7*</td>
<td>1138±20</td>
<td>197±3</td>
<td>3.12±0.07*</td>
<td>0.538±0.009*</td>
</tr>
<tr>
<td>Ben (5)+Val (15)</td>
<td>10</td>
<td>352±9*</td>
<td>1115±20</td>
<td>189±4</td>
<td>3.17±0.04*</td>
<td>0.538±0.009*</td>
</tr>
<tr>
<td>Ben (1)+Val (3)</td>
<td>9</td>
<td>345±8†</td>
<td>1114±24</td>
<td>184±8</td>
<td>3.24±0.07*</td>
<td>0.534±0.022*</td>
</tr>
</tbody>
</table>

BW indicates body weight; LV, left ventricular weight; RV, right ventricular weight; Low Na, 0.3% NaCl diet; High Na, 8% NaCl diet; Veh, vehicle treatment (control group); Ben (10), benazepril 10 mg·kg⁻¹·d⁻¹; Val (30), valsartan 30 mg·kg⁻¹·d⁻¹; Ben (5)+Val (15), combination of benazepril 5 mg·kg⁻¹·d⁻¹ and valsartan 15 mg·kg⁻¹·d⁻¹; and Ben (1)+Val (3), combination of benazepril 1 mg·kg⁻¹·d⁻¹ and valsartan 3 mg·kg⁻¹·d⁻¹. Values are mean±SEM. *P<0.01, †P<0.05 vs Veh.
higher LV ET-1 levels than those fed a low-salt diet (1085±71 versus 485±43 pg/g tissue, \(P<0.01\)). This increase in LV ET-1 levels was significantly suppressed only by the combination of benazepril 5 mg/kg and valsartan 15 mg/kg (812±50 pg/g tissue, \(P<0.05\)).

**Echocardiographic Analysis**

As shown in Figure 5, E/A in 16-week-old DS rats fed a high-salt diet was 5.4-fold greater than those fed a low-salt diet (7.63±0.75 versus 1.41±0.06, \(P<0.01\)). All drug treatments significantly prevented the increase in E/A (\(P<0.01\)). However, the normalization of E/A by combined benazepril 5 mg/kg and valsartan 15 mg/kg (1.86±0.20) was greater than that by any other drug treatments. Table 2 indicates that there was no difference in LV end-diastolic dimension, fractional shortening, LV end-diastolic volume, or LV ejection fraction among all groups of DS rats. Anterior and posterior wall thicknesses at end diastole in DS rats were increased by a high-salt diet, and this increase was not affected by any drug treatments.

**Urinary Catecholamine Excretions**

At 10 weeks of age, 24-hour urinary norepinephrine and epinephrine excretions were not different between DS rats fed a high-salt and a low-salt diet and were not affected by any drug treatments (data not shown). However, as shown in Figure 6, 14-week-old DS rats fed a high-salt diet had a 1.7-fold greater urinary norepinephrine excretion than those fed a low-salt diet (2.25±0.35 versus 1.33±0.10 \(\mu g/dL\), \(P<0.01\)). This increase in norepinephrine excretion was significantly and similarly prevented by all drug treatments. There was no difference in 24-hour urinary epinephrine excretion among all groups of DS rats at 14 weeks of age.

**Survival Rate**

Survival rate was analyzed at 20 weeks (140 days) after start of drug treatment (Figure 7). All vehicle-treated DS rats fed a high-salt diet died of congestive heart failure between 49 and 120 days. The Kaplan-Meier survival analysis showed that all drug treatments statistically significantly prolonged survival rate of DS rats, and there was no significant difference in improvement of survival among benazepril alone at 2 or 10 mg/kg, valsartan alone at 6 or 30 mg/kg, and combined benazepril 1 mg/kg and valsartan 3 mg/kg. However, the combination of benazepril 5 mg/kg and valsartan 15 mg/kg improved survival of DS rats more significantly than all monotherapies with benazepril 2 (\(P<0.01\)) or 10 (\(P<0.01\)) mg/kg or valsartan 6 (\(P<0.01\)) or 30 (\(P<0.05\)) mg/kg and the combination of benazepril 1 mg/kg and valsartan 3 mg/kg (\(P<0.05\)).

**Discussion**

Our present work provided solid experimental evidence that combination therapy with ACE inhibitor and ARB is more beneficial than each agent alone for treatment of heart failure.
It has been well established that DS rats fed 8% NaCl diet develop overt heart failure and die of congestive heart failure.\textsuperscript{19,20} Interestingly, the initiation of 8% NaCl diet in DS rats from 7 weeks of age produces diastolic heart failure with preserved systolic function.\textsuperscript{20} Although heart failure due to diastolic dysfunction often occurs in humans, the mechanism of diastolic heart failure is poorly understood.\textsuperscript{16,17} Despite the poor prognosis, a useful and specific therapeutic strategy for diastolic heart failure has not yet been established.\textsuperscript{24} Therefore, in this study, to investigate whether the combination of ACE inhibitor and ARB is beneficial for treatment of diastolic heart failure, we used DS rats fed a high-salt diet from 7 weeks of age.

As indicated by echocardiography, despite the lack of systolic dysfunction or LV dilatation, DS rats had significant diastolic dysfunction, as shown by the significant increase in E/A, and died of congestive heart failure. The present findings are in good agreement with the previous report.\textsuperscript{20} In this study, we found that either ACE inhibitor or ARB significantly improved E/A and prolonged survival in heart failure of DS rats. Notably, despite there being no additive hypotensive effect, the combination of ACE inhibitor and ARB improved E/A to a greater extent than monotherapy with either agent, being associated with more improvement of survival by the combination therapy (Figure 7). Thus, our present work supports the theory that combination therapy with these 2 agents is a useful therapeutic strategy for treatment of congestive heart failure.

In this study, we found that DS rats with diastolic dysfunction exhibited not only concentric LV hypertrophy but also myocyte phenotypic modulation, as shown by the upregulation of fetal genes such as ANP and BNP and the reciprocal downregulation of \(\alpha\)-MHC (adult isoform of MHC). Interestingly, the combination therapy suppressed LV ANP mRNA expression to a larger extent than monotherapy, suggesting that the combination therapy may be more effective for normalization of cardiac phenotypic modulation in pathological cardiac hypertrophy.

LV collagen contents were significantly increased in DS rats with diastolic dysfunction. Given that collagen accumulation is responsible for cardiac stiffness,\textsuperscript{25} diastolic dysfunction in DS rats seems to be explained at least in part by the increased LV collagen accumulation. It is noteworthy that only the combination of ACE inhibitor and ARB significantly decreased LV collagen contents in DS rats. This reduction of LV collagen contents seems to be due to the change in the synthesis rate or the degradation rate rather than the transcription rate, because LV collagen mRNA levels were comparably reduced by all drug treatments, and either the synthesis rate or the degradation rate plays an important role in the metabolism of LV collagen.\textsuperscript{26} Therefore, more improvement of heart failure by the combination therapy may be mediated in part by the reduction of LV collagen accumulation. However, further study is needed to demonstrate our proposal.

Iwanaga et al\textsuperscript{23} produced DS rats with systolic heart failure by starting a high-salt diet from 6 weeks of age and examined the role of LV ET-1 in systolic heart failure of DS rats. These investigators demonstrated that LV ET-1 plays a critical role in the development of systolic heart failure in DS rats fed a high-salt diet from 6 weeks of age. Furthermore, LV ET-1 is also involved in the development of heart failure induced by myocardial infarction or hemodynamic overload.\textsuperscript{27} Therefore, in the present study, we measured LV ET-1 levels in DS rats with diastolic LV dysfunction. We found that as in DS rats with the phenotype of systolic heart failure,\textsuperscript{23} LV ET-1 contents were significantly increased in DS rats with the phenotype of diastolic heart failure. Very importantly, this increase in LV ET-1 was significantly prevented only by the combination of ACE inhibitor and ARB. These findings, taken together with the fact that ET-1 has toxic effects on cardiac myocytes,\textsuperscript{28} induces cardiac fetal gene expression,\textsuperscript{29} and stimulates cardiac collagen synthesis,\textsuperscript{30} support the notion that the reduction of LV ET-1 levels by the combination therapy may be involved in the amelioration of heart failure.

Solid evidence indicates that Ang II directly stimulates LV ANP gene expression,\textsuperscript{31} ET-1 production,\textsuperscript{32} and collagen accumulation\textsuperscript{33} via \(\alpha\)1 receptor independently of its hypertensive effect, as reviewed.\textsuperscript{2} Therefore, in this study, the
mechanism underlying the greater suppression of LV ANP gene expression, ET-1 production, and collagen accumulation in DS rats by combined ACE inhibitor and ARB than by either agent alone may be explained by more potent inhibition of Ang II–mediated AT$_2$ receptor activation itself by the combination therapy. However, ACE inhibitor is well known to increase tissue bradykinin accumulation, and bradykinin has antigrowth effects and reduces vasomotor tone. Therefore, the possibility cannot be excluded that the accumulation of bradykinin by ACE inhibitor might participate in the present beneficial effects of the combination therapy in DS rats. Conversely, unlike ACE inhibitor, ARB increases circulating Ang II levels, leading to the stimulation of AT$_2$ receptor, which has antigrowth effects. However, unlike treatment with ARB alone, the combination with ACE inhibitor suppresses plasma Ang II elevation induced by ARB, indicating that AT$_2$ receptor activation caused by ARB alone is nullified by the combination with ACE inhibitor. Therefore, it is unlikely that AT$_2$ receptor might contribute to the beneficial effects of the combination therapy in the present study. However, further work is needed to elucidate more detailed mechanisms responsible for the beneficial effects of the combination therapy in heart failure.

**Study Limitations**

DS rats fed a high-salt diet are a useful and unique diastolic heart failure model. However, the characteristics of heart failure in DS rats are very complex, and several study limitations are raised. Because E/A can be affected by loading conditions as well as diastolic function, the improvement of E/A by the drug treatments might be partially mediated by the improvement of loading conditions. A high-salt diet significantly reduced body weight of DS rats, and this decrease was prevented by the drug treatments, which did not allow us to accurately estimate the effect of drug therapy on cardiac hypertrophy. DS rats develop renal dysfunction as well as heart failure. Therefore, the improvement of survival by the drug treatments might be partially due to the amelioration of renal dysfunction. Furthermore, a possible role of the sympathetic nervous system cannot be completely excluded in the present study, because urinary norepinephrine excretion in DS rats was significantly reduced by the drug treatments, and the sympathetic nervous system is responsible for the development of heart failure.

**Conclusions**

In conclusion, we examined the effects of ACE inhibitor, ARB, and their combination on cardiac hypertrophy, remodeling, gene expression, ET-1 levels, cardiac function, and survival rate in a rat diastolic heart failure model. Our present study provided evidence that either ACE inhibitor or ARB has beneficial effects on diastolic heart failure but that their combination has more beneficial effect than either agent alone. Thus, we propose that the combination of ACE inhibitor and ARB may be a potent therapeutic strategy for treatment of heart failure in humans.

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**References**


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