Original Article

Combination Treatment with a Calcium Channel Blocker and an Angiotensin Blocker in a Rat Systolic Heart Failure Model with Hypertension

Masashi NAMBA, Shokei KIM, Yumei ZHAN, Takafumi NAKAO, and Hiroshi IWAO

The mechanism and treatment of hypertensive systolic heart failure are not well defined. We compared the effect of an angiotensin-converting enzyme inhibitor (cilazapril, 10 mg/kg), an angiotensin receptor blocker (candesartan, 3 mg/kg), a calcium channel blocker (benidipine, 1, 3 or 6 mg/kg), and the same calcium channel blocker combined with renin-angiotensin blockers on systolic heart failure in Dahl salt-sensitive (DS) rats. DS rats were fed an 8% Na diet from 6 weeks of age and then subjected to the above drug treatments. Benidipine (1 mg/kg), cilazapril, and candesartan had compatible hypotensive effects and similar beneficial effects on cardiac hypertrophy, gene expression, and survival rate. The combination of benidipine with cilazapril or candesartan was found to have no additional beneficial effects on the above parameters, with the exception of a reduction in atrial natriuretic polypeptide gene expression. On the other hand, candesartan normalized serum creatinine, but serum creatinine was unaffected by either benidipine at 1 or 3 mg/kg or cilazapril. Further, the combined use of benidipine and either candesartan or cilazapril resulted in an additional reduction of urinary albumin excretion in DS rats. Thus systolic heart failure in DS rats is mainly mediated by hypertension, while renal dysfunction of DS rats is due to both hypertension and the AT1 receptor itself. These findings suggest that the combination of a calcium channel blocker with an AT1 receptor blocker or ACE inhibitor may be more effective in treating the renal dysfunction associated with systolic heart failure than monotherapy with either agent alone. However, further studies will be needed before reaching any definitive conclusion on the efficacy of this combination therapy in patients with heart failure.

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Key Words: hypertension, systolic heart failure, calcium blocker, angiotensin, combination therapy

Introduction

Accumulating experimental and clinical evidence indicates that angiotensin-converting enzyme (ACE) inhibitors and angiotensin II type 1 (AT1) receptor blockers (ARBs) are effective in treatment of cardiac hypertrophy and remodeling or congestive heart failure (1–3). In addition to blockers of the renin-angiotensin system, long-acting calcium channel blockers have recently been reported to have favorable ef-

fects on hypertension and other cardiovascular diseases. It is thus of great clinical importance to compare calcium channel blockers, ACE inhibitors and ARB with respect to their effects on cardiac hypertrophy and remodeling. Furthermore, in clinical treatment of hypertensives, combination therapy with different types of antihypertensive drugs is very frequently used in an effort to improve on the benefits of monotherapy.

Despite advances in pharmacological therapy for congestive heart failure, mortality due to heart failure remains high

From the Department of Pharmacology, Osaka City University Medical School, Osaka Japan.

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Address for Reprints: Shokei Kim, M.D., Ph.D., Department of Pharmacology, Osaka City University Medical School, 1–4–3 Asahi-machi, Abeno-ku, Osaka 545–8585, Japan. E-mail: kims@med.osaka-cu.ac.jp

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Fig. 1. Time course of blood pressure of Dahl-salt sensitive (DS) rats. DS rats were fed a high-salt diet from 6 weeks of age, and treated with vehicle (Ve), benidipine at 1, 3 and 6 mg/kg/day (Be(1), Be(3) and Be(6), respectively), candesartan at 3 mg/kg/day (Can), cilazapril at 10 mg/kg/day (Cil), benidipine (3 mg/kg/day) combined with candesartan (3 mg/kg/day) (Be(3) + Can), or benidipine (3 mg/kg/day) combined with cilazapril (10 mg/kg/day) (Be(3) + Cil). "Low" indicates DS rats fed a low-salt diet throughout the experiments. Values are the mean $\pm$ SEM ($n = 9$).

(4, 5). Dahl salt-sensitive rats (DS rats) fed an 8% NaCl diet from 6 weeks of age develop left ventricular (LV) systolic dysfunction and die of congestive heart failure, indicating that DS rats are a useful model of systolic heart failure (6–9). In the present study, we used this model to compare the therapeutic efficacy of the calcium channel blocker benidipine, the ACE inhibitor cilazapril, and the ARB candesartan cilexetil, as well the effects of the combined use of benidipine and either cilazapril or candesartan cilexetil.

Methods

Materials

Benidipine (10, 11) and candesartan cilexetil (12, 13) were obtained from Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan). Cilazapril was the gift of Eisai Co., Ltd (Tokyo, Japan).

Experimental Animals and Protocol

All procedures were in accordance with the guidelines for animal research of our institution. Dahl salt-sensitive (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 6 weeks of age. At 6 weeks of age, the diet was switched to an 8% NaCl diet, and the rats were divided into 8 groups that were respectively treated with 1) vehicle (0.5% carboxymethylcellulose solution), 2) benidipine (1 mg/kg/day), 3) benidipine (3 mg/kg/day), 4) benidipine (6 mg/kg/day), 5) candesartan cilexetil (3 mg/kg/day), 6) cilaza-

Fig. 2 Body weight, left ventricular (LV) weight and kidney weight in DS rats treated with each drug for 10 weeks. Abbreviations used are the same as in Fig. 1. Values are the mean $\pm$ SEM ($n = 9$ –10). * $p < 0.05$ vs. Ve.

pril (10 mg/kg/day), 7) benidipine (3 mg/kg/day) and candesartan cilexetil (3 mg/kg/day), and 8) benidipine (3 mg/kg/day) and cilazapril (10 mg/kg/day). We have previously reported that the present doses of cilazapril (10 mg/kg/day) and candesartan (3 mg/kg/day) were pharmacologically sufficient to exert a significant hypotensive effect, a significant inhibition of cardiac hypertrophy, or a significant improvement of renal impairment in hypertensive or diabetic rats (1, 12–17). All drugs were orally administered to DS rats by gastric gavage once a day until 16 weeks of age (*i.e.*, for 10 weeks). The systolic blood pressure of conscious rats was measured by the tail-cuff method at 4 to 5 h after each oral dosing. At 15 weeks of age, DS rats were housed individually in metabolic cages and 24-h urine was collected in a flask for measurement of urinary albumin excretion. 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 left ventricle was

Fig. 3. Cardiac brain natriuretic peptide (BNP), atrial natriuretic peptide (ANP) and transforming growth factor- β 1 (TGF- β 1) mRNA levels in DS rats treated with each drug for 10 weeks. Left ventricular (LV) BNP, ANP and TGF- β 1 mRNA levels were corrected for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels in individual rats. The mean value in the Low group is represented as 1. Abbreviations used are the same as in Fig. 1. Values are the mean $\pm$ SEM (n = 8–10). * $p < 0.05$ vs. Ve. NS, not significant.

separated from the atria and the right ventricle, and then these samples were weighed, frozen in liquid nitrogen and stored at -80°C until use.

RNA Preparation and Northern Blot Analysis

All procedures were performed as described in detail in our previous report (18). In brief, 20 μg of total RNA samples from individual left ventricles were subjected to 1% agarose gel electrophoresis, transferred to nylon membrane, and hybridized with [^{32}P] dCTP-labeled cDNA probe for atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), transforming growth factor- β 1 (TGF- β 1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The densities of an individual mRNA band were measured by using a bioimaging analyzer (BAS-2000; Fuji Photo Film Co., Tokyo, Japan).

Biochemical Measurement

Urinary albumin concentrations were measured by ELISA using anti-albumin antibody. Serum creatinine was measured by the kit.

Effects on Survival Rate

To examine the effects on survival, 6-week-old DS rats were fed an 8% NaCl diet and subjected to the drug treatments described above. The survival rate at 200 days after the start of treatment was then compared among groups.

Statistics

Results were expressed as the mean $\pm$ SEM. Statistical significance was determined by one way ANOVA followed by Duncan's multiple range test. Survival was analyzed by standard Kaplan-Meier analysis, log-rank test and χ^2 analysis. In all tests, values of $p < 0.05$ were considered to indicate statistical significance.

Results

Blood Pressure and Cardiac Weight

As shown in Fig. 1, DS rats fed a high-salt diet (8% NaCl) from 6 weeks of age progressively developed hypertension. All drug treatments significantly reduced the blood pressure of DS rats compared with vehicle treatment ($p < 0.01$). Throughout the treatment period, the hypotensive effects were comparable among the 3 groups treated with benidipine (1 mg/kg), candesartan (3 mg/kg) and cilazapril (10 mg/kg). Benidipine (3 mg/kg) produced a greater hypotensive effect than the above 3 treatments, but the combined use of benidipine (3 mg/kg) and candesartan (3 mg/kg) or cilazapril (10 mg/kg) did not augment this hypotensive effect. DS rats treated with benidipine (6 mg/kg) had lower blood pressure than any of the other groups ($p < 0.01$).

Figure 2 shows the body weight, left ventricular weight and kidney weight of DS rats after 10 weeks of drug treatment. The mean body weight of DS rats fed a high-salt diet was lower than that of rats fed a low-salt diet ($p < 0.01$), and all drug treatments significantly increased the body weight of DS rats. The LV weight in DS rats was significantly reduced by all drug treatments, but the combination of benidipine (3

Fig. 4. Twenty-four-hour urinary albumin excretion (UalbV) and serum creatinine in DS rats treated with each drug for 10 weeks. Abbreviations used are the same as in Fig. 1. Values are the mean $\pm$ SEM ($n = 8-10$). * $p < 0.05$ vs. Ve.

mg/kg) and candesartan or cilazapril did not enhance this reduction of cardiac weight. The increase in kidney weight of DS rats was significantly decreased by all drug treatments except for cilazapril.

Cardiac Gene Expression

As shown in Fig. 3, LV levels of BNP, ANP and TGF- β 1 mRNA in rats fed a high-salt diet were 4.2-fold, 33-fold and 1.9-fold ($p < 0.01$) larger than those in rats fed a low-salt diet. Comparable and significant reductions in LV BNP mRNA were achieved by benidipine (3 or 6 mg/kg) and by benidipine (3 mg/kg) in combination with candesartan or cilazapril. LV ANP mRNA levels were significantly reduced by all drug treatments. LV ANP mRNA levels in rats receiving benidipine in combination with candesartan or cilazapril were not significantly different from those in rats fed a low-salt diet, while those in rats subjected to benidipine (3 mg/kg) monotherapy were higher ($p < 0.05$) than those in rats fed a low-salt diet. LV TGF- β 1 mRNA levels were sig-

Fig. 5. Kaplan-Meier survival curves. DS rats fed a high-salt diet from 6 weeks of age were simultaneously treated with each drug. Abbreviations used are the same as in Fig. 1. The arrow indicates the start of drug treatment. Each group included 9 or 10 rats.

nificantly reduced only by the combination of benidipine (6 mg/kg) and candesartan.

Urinary Albumin Excretion and Serum Creatinine

UalbV was significantly larger in vehicle-treated DS rats fed a high-salt than in those fed a low-salt diet, and benidipine (6 mg/kg), or benidipine (3 mg/kg) combined with candesartan or cilazapril significantly reduced UalbV (Fig. 4). The increased serum creatinine levels were significantly reduced by benidipine (6 mg/kg) alone, candesartan alone, or benidipine (3 mg/kg) combined with candesartan or cilazapril.

Survival Rate

Survival rate was analyzed at 200 days after the start of drug treatment. All vehicle-treated DS rats fed a high-salt diet died at between 62 and 142 days after exhibiting tachypnea, labored respiration and loss of activity. The lungs of these vehicle-treated rats were edematous and weighed more than those of rats fed a low-salt diet (16.4 ± 1.6 vs. 4.2 ± 0.2 mg/g body weight; $p < 0.01$). The Kaplan-Meier survival analysis showed that all drug treatments statistically significantly prolonged the survival rate of DS rats ($p < 0.01$), and there was no significant difference in the improvement of survival among benidipine (1 mg/kg), candesartan and cilazapril (Fig. 5). There was no death in all other drug treatments until 200 days.

Discussion

It has been well established that DS rats fed an 8% Na diet develop not only hypertension but also overt congestive heart failure leading to death (7, 8). For example, administra-

tion of a high-salt diet in DS rats from 6 weeks of age leads to heart failure due to systolic cardiac function (8, 19). On the other hand, the initiation of an 8% Na diet in DS rats from 7 weeks of age produces diastolic heart failure with preserved systolic function (7). Thus DS rats exhibit different phenotypes of heart failure according to the starting point of the 8% NaCl diet.

In this study, we used a systolic heart failure model of DS rats, because the impact of high blood pressure or the renin-angiotensin system on systolic heart failure in this model is not fully defined. The onset of heart failure in vehicle-treated DS rats was supported by the observation of tachypnea, labored respiration, loss of activity, and pulmonary congestion. In this work, benidipine (1 mg/kg), candesartan and cilazapril showed similar hypotensive effects on DS rats throughout treatment. There were no significant differences among these three drugs with respect to LV weight, LV BNP and ANP gene expression or survival rate. These results, together with the observations that the dose-dependent lowering of hypertension by benidipine was associated with the dose-dependent improvements in LV weight, LV gene expressions and survival rate, show that blood pressure-lowering effects rather than angiotensin blockade are important to the improvement of cardiac hypertrophy and dysfunction in DS rats with systolic heart failure. On the other hand, LV TGF- β 1 expression was significantly suppressed by candesartan but not by cilazapril or benidipine (1 or 3 mg/kg), suggesting that AT1 receptors contributed to the LV TGF- β 1 expression in DS rats. And although we did not analyze the difference in TGF- β 1 expression between candesartan and cilazapril in the present study, we previously observed a difference in TGF- β 1 expression by these two drugs in stroke-prone spontaneously hypertensive rats (15).

Combination therapy of antihypertensive drugs is very frequently performed in hypertensive patients. However, it has not been determined whether the combination of a calcium channel blocker and a renin-angiotensin blocker is more effective for ameliorating systolic heart failure than the use of either drug alone. Therefore, we here examined the effects on cardiac hypertrophy, gene expression, and survival rate following combination treatment with a calcium channel blocker and a renin-angiotensin blocker, and compared the results with those for the respective monotherapies. With the exception of a suppressive effect on LV ANP mRNA expression, we found no evidence that this combination treatment was more effective than the use of either drug alone. However, further studies will be needed to clarify the significance of this combination therapy in heart failure, since the effects of candesartan and cilazapril were examined only at a single dose in this study.

Renal dysfunction plays an important role in the pathophysiology of systolic heart failure. Of note, candesartan, but not cilazapril or benidipine (1 or 3 mg/kg), normalized serum creatinine, indicating the impact of AT1 receptor on renal dysfunction in DS rats. Furthermore, the combination

of benidipine with candesartan or cilazapril showed additive beneficial improvement of urinary albumin excretion. Thus, unlike cardiac hypertrophy or systolic dysfunction, the renal dysfunction in DS rats is mediated by the AT1 receptor as well as by hypertension. We cannot fully explain why cilazapril failed to decrease serum creatinine in the present study. However, this result was not likely to have been due to use of an insufficient dose of cilazapril, since we have previously reported that 10 mg/kg of cilazapril significantly improved renal function in other hypertensive or diabetic rat models (14, 16).

Interestingly, the initiation of an 8% NaCl diet in DS rats from 7 weeks of age produces diastolic heart failure with preserved systolic function (7, 20). However, it remains to be determined whether the present observations on systolic heart failure of DS rats can be applied to diastolic heart failure of DS rats. Further studies will be needed to determine the possible relative role of blood pressure and AT1 receptors in the pathophysiology of diastolic heart failure.

In conclusion, this work showed that high blood pressure rather than the AT1 receptor had the greatest impact on systolic heart failure in DS rats. On the other hand, renal dysfunction is due to not only hypertension but also the AT1 receptor, and thus the combination of a calcium blocker and a renin-angiotensin blocker would seem to be useful for treatment of renal dysfunction. However, further study will be needed to fully clarify the significance of this combination therapy in heart failure.

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