Beneficial Effect of Candesartan on Rat Diastolic Heart Failure

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Abstract. In this study, we examined whether an angiotensin II type 1 (AT₁)-receptor blocker improves diastolic heart failure (DHF) in Dahl salt-sensitive (DS) rats. DHF was prepared by feeding DS rats on 8% NaCl diet from 7 weeks of age. DHF was estimated with echocardiography by measuring E velocity / A velocity (E/A) of left ventricular inflow. DS rats with established DHF were orally given candesartan (1 mg/kg per day) or vehicle. After 13 days of treatment, candesartan significantly improved DHF, as shown by the reduction of E/A from 4.49 ± 1.04 to 1.98 ± 0.54 (P<0.05) and prolonged survival rate more than the vehicle. Cardiac fibrosis, apoptosis, and gene expression were estimated by Sirius Red-staining, TUNEL-staining, and Northern blot analysis, respectively. The improvement of DHF by candesartan was accompanied by the decrease in cardiac hypertrophy, fibrosis, and apoptosis, and the reduction of gene expression of brain natriuretic peptide, collagen I, and monocyte chemoattractant protein-1. Moreover, candesartan decreased cardiac inflammatory cells and reactive oxygen species, estimated by counting ED-1-positive cells and the measurement of 4-hydroxy-2-nonenal staining, respectively. These results indicate that candesartan can improve diastolic dysfunction and may slow the progression of cardiac remodelling in DS rats with established DHF.

Keywords: AT₁-receptor blocker, diastolic heart failure, fibrosis, hypertrophy, apoptosis

Introduction

Epidemiological studies have established that 30 to 50 percent of patients with heart failure, despite preserved ejection fraction without valve disease, are attributed to left ventricular (LV) diastolic dysfunction (1). That is defined as diastolic heart failure (DHF). Hypertensive heart disease is the major risk factor of DHF (2). However, the therapeutic strategy of DHF has not been fully established (3).

Multiple lines of experimental and clinical studies indicate that angiotensin II induces not only hypertension but also various cardiac diseases (4 – 7). Furthermore, a growing body of evidence show that angiotensin II type 1 (AT₁)-receptor blockers inhibit cardiac hypertrophy and remodeling, and prevent progression of systolic heart failure, thereby reducing cardiac morbidity and mortality (7 – 9). However, the effect of AT₁-receptor blocker on DHF remains to be determined.

Dahl salt-sensitive (DS) rats, fed a high-salt diet from 7 weeks of age, develop hypertension followed by overt DHF with increased LV filling pressure and pulmonary congestion (10 – 13). Thus, DS rats are regarded as a useful model of human DHF. Using this model, we and other groups of investigators have demonstrated that AT₁-receptor blockers or angiotensin-converting enzyme inhibitors prevent the onset of DHF, when the therapy is initiated before the onset of DHF (14 – 16). However, no information is available on the effects of AT₁-receptor blockers on established DHF. In this study, we examined whether or not candesartan is effective on DS rats with established DHF. In the present study, we obtained evidence that candesartan exerts beneficial effects on DHF of DS rats when its administration is
started at the established DHF stage.

**Materials and Methods**

**Experimental animals and protocol**

All procedures were in accordance with institutional guidelines for animal research. DS rats (DIS/Eis; Eisai, Tokyo) 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, the diet of DS rats was switched to an 8% NaCl (high-salt) diet. Systolic blood pressures of conscious rats were periodically measured by the tail-cuff method (BP98A; Softron, Tokyo) at 4 to 5 h after oral dosing. Cardiac function was periodically monitored by echocardiography as described below. After DS rats were found to have remarkable diastolic dysfunction, they were separated into 2 groups and began to be orally given candesartan (1 mg/kg per day) or vehicle (0.5% carboxymethyl-cellulose solution) by gastric gavage once a day for 13 days. There was no significant difference between vehicle and candesartan groups, with regard to the age when the treatment was started (21.4 ± 0.6 vs 22.2 ± 0.7 weeks of age). We compared survival between the vehicle group and candesartan group throughout the treatment. All animals were carefully monitored, and the number of dead rats was recorded every day. After the administration for 13 days, echocardiography was performed as described below. The hearts were immediately excised, and the left ventricle was separated from the atrium and the right ventricle. Furthermore, other transverse sections were stained with the lectin for measurement of cardiomyocyte cross-sectional area, with the collagen-specific Sirius Red for measurement of interstitial fibrosis and perivascular fibrosis of coronary artery and with the TdT-mediated dUTP nick-end labeling (TUNEL) method for detection of apoptosis (20).

**Echocardiographic study**

Transthoracic echocardiographic studies were performed on DS rats with SONOS 5500 (Philips Medical Systems Corp., Best, Netherlands), as previously described in detail (17). In brief, rats were anesthetized with ketamine HCl and xylazine. The two-dimensional short-axis view of the left ventricle and M-mode tracings were recorded through the intraventricular septum and posterior LV walls at the papillary muscle level to measure LV end-diastolic dimension, fractional shortening, and LV ejection fraction. Pulse-wave Doppler spectra [E and A wave velocity and deceleration time (Dct)] of mitral inflow were recorded from the apical 4-chamber view, with the sample volume placed near the tips of the mitral leaflets. All Doppler spectra were recorded on paper at 100 mm/s and analyzed off-line. E velocity is the peak early diastolic filling velocity and A velocity is the peak filling velocity on atrial contraction. The more the left ventricular end diastolic pressure increases because of diastolic dysfunction, the more E/A increases (18, 19). Tissue Doppler imaging was used to measure myocardial tissue velocities at the septal mitral annular regions in the apical 4-chamber views. The early diastolic myocardial velocity at the mitral annular regions (Ea) is measured as an index of diastolic function. The more the left ventricular end diastolic pressure increases due to diastolic dysfunction, the more E/Ea increases (18, 19). Echocardiographic studies showed that DS rats exhibited diastolic dysfunction characterized by E/A>2 of LV inflow with restrictive pattern, with preserved systolic function, during 20–25 weeks of age.

**Northern blot analysis**

Northern blot analysis of total RNA samples from individual left ventricles were carried out with the probes for rat brain natriuretic peptide, collagen type I, rat monocyte chemoattractant protein-1, and rat glycer-aldehyde-3-phosphate dehydrogenase (15).

**Immunohistochemistry**

Three-µm-thick sections of left ventricles, embedded in paraffin, were stained with the lectin for measurement of cardiomyocyte cross-sectional area, with the collagen-specific Sirius Red for measurement of the interstitial fibrosis and perivascular fibrosis of coronary artery and with the TdT-mediated dUTP nick-end labeling (TUNEL) method for detection of apoptosis (20). Furthermore, other transverse sections were stained with mouse anti-rat ED-1 monoclonal antibody (1:100 dilution, Ki-M2R; BMA Biomedicals, Ltd., Augst, Switzerland) (21) to count macrophages or with mouse monoclonal anti 4-hydroxy-2-nonenal (HNE) antibody (22) (1:50 dilution; NOF Medical Department, Tokyo) to measure HNE stained area.

**Statistical analyses**

All data are presented as the mean ± S.E.M. Statistical significance was determined with one-way ANOVA, two-way ANOVA, and the Duncan multiple range test using Super ANOVA (Abacus Concepts, Inc., Berkeley, CA, USA). Survival rate was analyzed by the standard Kaplan-Meier analysis with Breslow-Gehan-Wilcoxon test and χ² analysis. In all tests, differences were considered statistically significant at a value of P<0.05.
Results

Blood pressure

DS rats, fed a high-salt diet from 7 weeks of age, progressively developed hypertension with time. There was no significant difference in blood pressure between vehicle- and candesartan-treated groups (206 ± 2 vs 204 ± 3 mmHg) before the treatment. Blood pressure before and after candesartan treatment was 204 ± 3 and 196 ± 5 mmHg, respectively, and there was no significant difference in blood pressure between before and after candesartan treatment.

Survival rate

As shown in Fig. 1, survival rate was analyzed during the administration (candesartan group, n = 13 vs vehicle (Fig. 1. Kaplan-Meier survival curves. DS rats, fed a high-salt diet, were treated with vehicle (Veh) or candesartan (1 mg/kg per day) (Can).

![Fig. 2. Effect of candesartan on cardiac function estimated by echocardiography. Echocardiography was performed before (pre) and after (post) 13 days of drug treatment. Upper panels show representative M-mode of papillary level of left ventricle in a long axis view (A), representative Pulse-wave Doppler spectra (E and A velocity) of mitral inflow that was recorded from the apical 4-chamber view (B), and the representative tissue Doppler imaging that was myocardial tissue velocity at the septal mitral annular regions (C). Lower graphs show the quantitative analyses of IVS thickness, EF, FS, E/A, Dct, and E/Ea. Abbreviations used: IVS, intraventricular septum; EF, ejection fraction; FS, fractional shortening; E/A, the ratio of peak early diastolic filling velocity and peak filling velocity on atrial contraction; Dct, deceleration time of early diastolic filling wave; E/Ea, the ratio of peak early diastolic filling velocity and the early diastolic myocardial velocity at the mitral annular regions. Other abbreviations are the same as the Fig. 1 legend. Values are the mean ± S.E.M. (Veh: n = 9, Can: n = 11). *P<0.05 vs Veh, †P<0.01 vs Veh. †P<0.01 vs pre.]}
After 13 days of the treatment, 11 of 13 DS rats survived in the candesartan-treated group, while only 9 of 17 rats survived in the vehicle-treated group. The Kaplan-Meier survival analysis showed that candesartan prolonged survival rate more than vehicle ($P<0.05$). The cause of death of DS rats was congestive heart failure accompanied by massive pulmonary edema, as estimated by autopsy. As vehicle-treated DS rats exhibited severe heart failure after 13 days of the treatment, we evaluated the effect of 13 days of candesartan treatment in this study.

Doppler echocardiography

After 13 days of treatment, DS rats were subjected to echocardiography. As shown in Fig. 2, candesartan significantly slowed the progression of cardiac hypertrophy, as estimated by IVS thickness. Moreover, candesartan reduced E/A from $4.0 \pm 0.5$ to $2.0 \pm 0.5$ ($P<0.05$) and E/Ea from $46.8 \pm 4.4$ to $31.7 \pm 4.4$ ($P<0.05$) and increased Dct from $30.4 \pm 2.2$ to $38.4 \pm 2.2$ ($P<0.05$). On the other hand, candesartan did not affect EF or FS in DS rats.

Structural characteristics

As shown in Fig. 3, DS rats, fed a high salt diet, exhibited prominent cardiac hypertrophy and cardiac fibrosis, as shown by larger left ventricular weight, larger cross sectional area, and more interstitial fibrosis ratio compared with those fed a low salt diet. Candesartan treatment significantly slowed the progression of cardiac hypertrophy ($P<0.05$) and fibrosis ($P<0.05$) compared with vehicle.

Cardiac gene expression

As shown in Fig. 4, LV brain natriuretic peptide, collagen I, and monocyte chemoattractant protein-1 mRNA levels of the vehicle group were significantly larger than those of the low-salt diet group. Those values in the candesartan group were smaller than those in the vehicle group.

Effects of candesartan on cardiac apoptosis, inflammatory cells, and oxidative stress in the left ventricles of DS rats

As shown in Fig. 5, the number of TUNEL-positive nuclei and ED-1 positive cells, and HNE ratio in the

![Fig. 3. Cardiac hypertrophy and fibrosis of 3 groups of DS rats. A: Upper panels show representative images of light micrographs of cross section of cardiac midportion. Original magnification, ×1.25 (bar = 2.0 mm). The lower bar graph indicates LV weight corrected for body weight. B: The upper and lower panels show representative images of fluorescence micrographs of LV free-wall sections and LV cardiomyocyte cross-sectional area, respectively. Original magnification, ×400 (bar = 100 µm). C: The top panels show representative images of light micrographs of Sirius Red-stained LV sections. Original magnification, ×400 (bar = 100 µm). The bottom bar graph shows quantitative interstitial fibrosis ratio. Each bar represents the mean ± S.E.M. (Low: n = 6, Veh: n = 9, Can: n = 11). Low: DS rats fed low salt diet. Other abbreviations are the same as the Fig. 1 legend. *$P<0.05$, †$P<0.01$.](image-url)
Fig. 4. Cardiac hypertrophy- and fibrosis-related gene expression in DS rats. Left top panel shows representative autoradiograms of LV mRNAs for brain natriuretic peptide, collagen I, monocyte chemoattractant protein-1, and glyceraldehyde-3-phosphate dehydrogenase. Bar graph shows each mRNA value, corrected for glyceraldehyde-3-phosphate dehydrogenase mRNA value. Mean values in the low salt diet group is represented as 1. Each bar represents the mean ± S.E.M. (Low: n = 6, Veh: n = 9, Can: n = 11). Abbreviations are the same as the Fig. 3 legend. *P < 0.05, #P < 0.01.

Fig. 5. Cardiac TUNEL staining, immunohistochemistry with anti-ED-1 antibody and HNE staining. A: Top panels show representative images of light micrographs of TUNEL-positive nuclei that show cardiomyocyte apoptosis (Arrows). Original magnification, ×800 (bar = 100 µm). The bottom bar graph shows the number of TUNEL-positive nuclei corrected for total LV cardiomyocyte area. B: Top panels show representative images of light micrographs of LV ED-1-positive cells (monocytes/macrophage) (Arrows). Original magnification, ×400 (bar = 100 µm). The bottom bar graph shows the number of ED-1-positive cells for total LV cardiomyocyte area. C: The top panels show representative images of light micrographs of HNE staining in 3 groups. Original magnification, ×400 (bar = 100 µm). The bottom graph shows the ratio of HNE positive staining. Each bar represents the mean ± S.E.M. (Low: n = 6, Veh: n = 9, Can: n = 11). Abbreviations are the same as the Fig. 3 legend. *P < 0.05, #P < 0.01.
left ventricle from vehicle group were greater than those of the low salt group ($P<0.05$). The values in the candesartan group were smaller than those in the vehicle group.

**Effects of candesartan on perivascular fibrosis and coronary arterial thickening in the left ventricles of DS rats**

As shown in Fig. 6, we examined the effects of candesartan on perivascular fibrosis and coronary arterial thickening for large ($100\ \mu m < \text{diameter}$) and small ($50\ \mu m < \text{diameter} < 100\ \mu m$) coronary arteries in the left ventricle. In both larger and small coronary arteries, perivascular fibrosis and coronary arterial thickening in vehicle group were higher than those in low salt group. The values in candesartan group were smaller than those in the vehicle group.

**Discussion**

DS rats, fed a high salt diet, are regarded as a useful model of DHF. In this study, we first examined whether an AT1-receptor blocker (candesartan) is effective in DS rats that already exhibit established DHF, and we found that candesartan significantly slowed the progression of cardiac hypertrophy and remodeling and improved cardiac diastolic dysfunction and survival rate. Furthermore, the improvement of DHF by candesartan might be associated with the attenuation of cardiac fibrosis, inflammation, oxidative stress, and apoptosis.

The treatment with candesartan significantly decreased the progression of the interstitial and perivascular fibrosis in DS rats. Fibrosis, which mainly consists of interstitial fibrosis and perivascular fibrosis, is the remodeling characteristic of the failing heart. Accumulation of collagen type I, the main fibrillar collagen found in cardiac fibrosis, stiffens the ventricles and impedes both contraction and relaxation. Furthermore, fibrosis results in the decrease of capillary density and an increased oxygen diffusion distance that can lead to hypoxia of myocytes (10). Thus, fibrosis profoundly aggravates myocyte metabolism and ventricular dia-

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![Fig. 6. Coronary arterial perivascular fibrosis in DS rats. A: Upper panels show representative images of light micrographs of cross section of coronary artery from Low, Veh, and Can. Original magnification, ×200 (bar = 100 µm). The bar graph show the percentage of coronary arterial perivascular fibrosis area (B) and coronary arterial media wall area (C) corrected for the lumen area. Each bar represents the mean ± S.E.M. Abbreviations are the same as the Fig. 3 legend. *$P<0.05$, †$P<0.01$.](image-url)
stolic function (23). Therefore, in this study, the improvement of diastolic function by candesartan seems to be mediated at least in part by the attenuation of LV collagen accumulation.

Cardiac inflammation, apoptosis, and oxidative stress are known to exacerbate cardiac function. Monocyte chemoattractant protein-1 and monocyte/macrophage contribute to inflammatory changes and pathogenesis of heart failure (24, 25). In congestive heart failure, the loss of cardiomyocytes occurred via the increase in apoptosis (26, 27), leading to deterioration of cardiac function. The previous data showed that cardiac function deteriorated in the process of apoptosis (28). Therefore, to elucidate the possible mechanism underlying the improvements of DHF in DS rats by candesartan, we examined the effect of candesartan on cardiac monocyte chemoattractant protein-1 mRNA and monocyte/macrophage infiltration, cardiac apoptosis, and oxidative stress in the heart. The increase of monocyte chemoattractant protein-1 mRNA in DS rats, fed high-salt diet, was suppressed by candesartan, being accompanied by the reduction of monocyte/macrophage infiltration. Thus, the suppression of inflammatory changes by candesartan might be involved in the improvement of cardiac fibrosis by candesartan. TUNEL staining indicated that the increase in cardiomyocyte apoptosis in DS rats was lessened by candesartan. Moreover, the results on the HNE staining showed that the increase of cardiac oxidative stress in DS rats is significantly decreased by candesartan. These observations suggest that the amelioration of cardiac inflammation, apoptosis, and oxidative stress might explain the improvement of DHF by candesartan.

In this study, candesartan did not apparently reduce blood pressure in DS rats compared with the pretreatment. However, it cannot be excluded that the beneficial effect of candesartan in DS rat with established DHF might be at least in part mediated by its hypotensive effect. Furthermore, in this study, the effect of candesartan was not compared between before and after the treatment, except for blood pressure and echocardiographic assessment. Therefore, the present study did not allow us to determine whether candesartan only prevented further progression of cardiac remodeling or reversed cardiac remodeling. Further study is needed to elucidate these unresolved issues.

The candesartan in heart failure-assessment of mortality and morbidity (CHARM)-Preserved Trial suggested beneficial effects of candesartan in patients with DHF, as shown by the reduction of the number of hospital admissions for heart failure by candesartan (29). Taken together with this clinical evidence, our present results support the notion that an AT₁-receptor blocker may be a useful therapeutic agent for DHF, although further experiments are needed to elucidate more detailed mechanisms responsible for the beneficial effects of the AT₁-receptor blocker on the end stage of DHF.

References