AT1 Receptor Blocker Added to ACE Inhibitor Provides Benefits at Advanced Stage of Hypertensive Diastolic Heart Failure

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Abstract—Diastolic heart failure (DHF) has become a social burden; however, evidences leading to its therapeutic strategy are lacking. This study investigated effects of addition of angiotensin II type 1 receptor blocker (ARB) to angiotensin-converting enzyme inhibitor (ACEI) at advanced stage of DHF in hypertensive rats. Dahl salt-sensitive rats fed 8% NaCl diet from age 7 weeks served as DHF model, and those fed a normal chow served as control. The DHF model rats were arbitrarily assigned to 3 treatment regimens at age 17 weeks: ACEI (temocapril 0.4 mg/kg per day), combination of ACEI (temocapril 0.2 mg/kg per day) with ARB (olmesartan 0.3 mg/kg per day), or placebo. At age 17 weeks, this model represents progressive ventricular hypertrophy and fibrosis, relaxation abnormality, and myocardial stiffening. Data were collected at age 20 weeks. As compared with the monotherapy with ACEI, the addition of ARB induced more prominent suppression of ventricular hypertrophy and fibrosis, leading to suppression of myocardial stiffening, improvement of relaxation, and inhibition of hemodynamic deterioration. Such benefits were associated with greater decreases in reactive oxygen species (ROS) generation, macrophage infiltration, and gene expression of transforming growth factor (TGF)-β1 and interleukin (IL)-1β, but not with changes in gene expression of monocyte chemoattractant protein (MCP)-1 and tumor necrosis factor (TNF)-α. Thus, ARB added to ACEI provides more benefits as compared with ACEI alone in DHF when initiated at an advanced stage. The additive effects are likely provided through more prominent suppression of ROS generation and inflammatory changes without effects on expression of MCP-1 and TNF-α. (Hypertension. 2004;43:686-691.)

Key Words: diastole ■ angiotensin II ■ angiotensin-converting enzyme ■ heart failure ■ oxidative stress

Occurrence of congestive heart failure despite preserved ejection fraction is attributed to left ventricular (LV) diastolic dysfunction and is termed diastolic heart failure (DHF). It consists of a high proportion of patients with congestive heart failure, and its major underlying cardiovascular disease is a hypertensive heart disease.1,2 Despite the social burden of DHF, its therapeutic strategy has not been established.

We have demonstrated that Dahl–Iwai salt-sensitive rats fed 8% NaCl from age 7 weeks present hypertension followed by compensatory LV hypertrophy with LV relaxation abnormality at approximately age 13 weeks; further progression of LV hypertrophy and development of LV fibrosis with LV relaxation abnormality and myocardial stiffening at approximately age 17 weeks; and overt DHF with increased LV filling pressure and pulmonary congestion at approximately age 20 weeks.3,4 Using this model, our and other experimental studies demonstrated preventive effects of angiotensin II type 1 receptor blocker (ARB), angiotensin-converting enzyme inhibitor (ACEI), and their combination when initiated before the onset of LV diastolic dysfunction (at age 7 or 8 weeks).5,6 However, therapeutic effects of any medication in DHF remain to be clarified when initiated at an advanced stage with LV diastolic dysfunction and structural alterations.

A few retrospective studies demonstrated better prognosis in association with the prescription of ACEI in patients with DHF.7,8 Recent clinical trials showed benefits of an addition of ARB to ACEI in patients with systolic heart failure.9,10 The Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity (CHARM)-Preserved trial reported that ARB reduced hospitalization for worsening of DHF.11 However, the additive effects of ARB to ACEI in DHF remain to be clarified, because ACEI was used in <20% of patients in the trial. This study aimed to investigate effects of addition of ARB to ACEI, independent of their antihypertensive effects in a hyper-
tensive DHF model when initiated after the appearance of LV diastolic dysfunction and structural alterations.

Methods

Subjects
Male Dahl salt-sensitive rats (DIS/Eis; Eisai, Tokyo, Japan) fed 8% NaCl chow from age 7 weeks served as DHF model and were randomized at age 17 weeks to untreated rats (untreated group, n = 6), rats treated with ACEI and ARB (temocapril 0.2 mg/kg per day, olmesartan 0.3 mg/kg per day; courtesy of Sankyo Co. Ltd; combination group, n = 6), or with ACEI (temocapril 0.4 mg/kg per day; ACEI group, n = 6). LV diastolic dysfunction and structural alterations.

Hemodynamic Studies
The rats were anesthetized with ketamine HCl (50 mg/kg) and xylazine HCl (10 mg/kg), and echocardiographic and LV pressure recordings were obtained to determine LV geometry, LV end-diastolic pressure, time constant of LV relaxation (Tau), and myocardial stiffness constant at age 20 weeks.12,15,16 The schedule was based on the fact that this DHF model presents pulmonary congestion with increased LV filling pressure at approximately age 20 weeks.3

Tissue Sampling
After the hemodynamic study, blood was sampled for measurement of serum creatinine, and the lung and the heart were harvested and weighed. LV weight corrected for body weight was determined as LV mass index.13 LV samples for measurement of mRNA and protein levels and in vitro zymography were immediately placed in liquid nitrogen and stored at −80°C. Samples for immunohistochemistry were embedded in Tissue-Tek OCT compound (Sakura Fine-technical Co; Tokyo, Japan) and frozen on dry ice. The other section was immersed in a cold 4% paraformaldehyde solution for 16 to 24 hours and used for Azan Mallory stain to determine the percent area of fibrosis, as previously described.3

Quantitative Reverse-Transcription Polymerase Chain Reaction Analysis
Real-time TaqMan reverse-transcription polymerase chain reaction was performed.12,15 The sequences of oligonucleotides used as forward primers, reverse primers, and TaqMan probes for type I collagen, type III collagen, transforming growth factor (TGF)-β1, and glyceroldehyde 3-phosphate dehydrogenase (GAPDH) were previously reported.12,15,16 Those for the other measured factors were: interleukin (IL)-1β, forward primer 5′-GCAGCATCTCGAC-AAGAGCTT-3′; reverse primer 5′-TCACCTGCTGCACTACT-3′; and monocyte chemotactic protein ( MCP)-1, forward primer 5′-CGGCTGCTCTCAAGGACC-3′; reverse primer 5′-GTGGACGCTCTCAAGGACC-3′. Each mRNA level was corrected for GAPDH mRNA level.

Western Blotting and In Vitro Gelatin Zymography
Western blot analysis of sarcoplasmic reticulum calcium (2+-) ATPase 2a (SERCA2a), phospholamban, and Ser16-phosphorylated phospholamban and in vitro gelatin zymography were performed as previously described.12,15,16

Immunohistochemistry
Cryostat transverse sections were stained using mouse anti-rat macrophages monoclonal antibody (1:50 dilution, Ki-M2R; BMA Biomedicals Ltd, Augst, Switzerland) or mouse monoclonal anti-4-hydroxy-2-nonenal (HNE) antibody (1:50 dilution; NOF Medical Department, Tokyo, Japan) as previously described.15

Statistical Analysis
Results are expressed as mean±SEM. Differences among groups were assessed using 1-factor ANOVA and Fisher protected least significant difference test; P<0.05 was considered statistically significant.

Results
Effect on Hemodynamics and LV Structural Characteristics
All echocardiographic and hemodynamic data at age 20 weeks were summarized in the Table. The untreated rats represented increases in LV end-diastolic pressure and lung weight, indicating the presence of pulmonary congestion caused by congestive heart failure.3 Such hemodynamic deterioration was associated
with increases in LV mass index, area of fibrosis, myocardial stiffness constant, and Tau, but endocardial and mid-wall fractional shortenings and LV end-diastolic dimension were not different from those of the control rats. These characteristics are compatible with those of DHF.

Neither administration of temocapril nor combined administration of olmesartan and temocapril affected blood pressure, endocardial and mid-wall fractional shortenings, and LV end-diastolic dimension. Although LV mass index was not different between the ACEI and untreated groups, administration of temocapril significantly decreased the area of fibrosis and tended to decrease Tau, myocardial stiffness constant, LV end-diastolic pressure, and lung weight. Addition of olmesartan significantly decreased LV mass index and Tau as compared with the untreated rats. In addition, the combination further decreased area of fibrosis and myocardial stiffness constant as compared with the monotherapy with temocapril. LV end-diastolic pressure and lung weight in the combination group were significantly lower than in the untreated group. This DHF model was associated with an increase in serum creatinine level at age 20 weeks as previously described, but serum creatinine level did not change with either therapeutic regimen.

Effects on Calcium Regulatory Proteins
In the untreated rats, phosphorylation level of phospholamban decreased without significant changes in protein levels of SERCA2a and phospholamban (Figure 1). Administration of temocapril attenuated the decrease in phosphorylation level of phospholamban, and addition of olmesartan completely reversed the level.

Effects on Regulatory System of Extracellular Matrix
The untreated rats represented increases in type I and type III collagen mRNA levels (Figure 2) and 72 kDa gelatinase (matrix metalloproteinase-2) activity (Figure 3). Administr-
tion of temocapril tended to decrease type I collagen mRNA level and significantly decreased type III collagen mRNA level and the 72 kDa gelatinase activity. Addition of olmesartan decreased type III collagen mRNA to a level similar to the monotherapy with temocapril but provided a significant decrease in type I collagen mRNA. The 72-kDa gelatinase activity decreased further with addition of olmesartan.

Effects on Inflammatory Changes and Reactive Oxygen Species
In the untreated rats, gene expression of IL-1β, TGF-β1, and MCP-1 was enhanced as compared with the control rats (Figure 2). TNF-α mRNA level was not different between the control and untreated rats. The immunohistochemical study revealed increases in macrophage infiltration (Figure 4) and HNE generation (Figure 5), a marker of reactive oxygen species (ROS) production, in the untreated rats.

Administration of temocapril attenuated the macrophage infiltration and decreased the HNE staining but did not alter gene expression of IL-1β, TGF-β1, TNF-α, and MCP-1. Addition of olmesartan induced further suppression of the macrophage infiltration and the HNE staining. In association with such changes, the TGF-β1 mRNA level significantly decreased, and the IL-1β mRNA level tended to decrease (P=0.08 versus the untreated group) without changes in gene expression of TNF-α and MCP-1.

Discussion
The combination therapy with ARB and ACEI blocked the progression of ventricular fibrosis and hypertrophy, even without reduction in blood pressure, when the medications were initiated at the advanced stage of DHF with LV fibrosis and hypertrophy, relaxation abnormality, and myocardial stiffening. The effects on LV fibrosis were attributed to inhibition of collagen synthesis rather than to enhancement of collagen degradation and were likely to result in the attenuation of myocardial stiffness. The reversal of Ser16-phosphorylated phospholamban level in association with the attenuation of LV hypertrophy may explain the improvement of LV relaxation. The combination therapy with ACEI provided less benefits to LV structure and function than did the combination therapy.

Renin-angiotensin system is known to promote ventricular fibrosis and hypertrophy principally through angiotensin II type I receptor. Recent clinical studies supported this concept. Because angiotensin II production independent of ACE exists, ARB is expected to block angiotensin II action more than ACEI. Previous experimental and clinical studies reported additional benefits of the combination therapy in systolic heart failure as compared with the monotherapy with ACEI. The current study expanded those previous studies by demonstrating that this concept can be applied to DHF as well as systolic heart failure. Kim et al showed that
the combination achieved more preventive benefits than a monotherapy in the same hypertensive DHF model when the medications were initiated before the appearance of hypertension, ventricular structural abnormalities, and diastolic dysfunction.6 The current study extended their finding and demonstrated that additive benefits of the combination can be achieved independent of antihypertensive effects, even if initiated after the appearance of structural abnormalities and diastolic dysfunction of the left ventricle. The CHARM-Preserved trial suggested beneficial effects of ARB in patients with DHF, but ACEI was combined in <20% of the study subjects.11 The current results permit further studies to elucidate therapeutic efficacy of the combination therapy with ACEI and ARB in DHF patients.

Macrophage infiltration plays an important role in the pathogenesis of hypertensive systolic heart failure.28–31 In this study, the medication-induced effects on macrophage infiltration were compatible with those on cardiac structural remodeling and diastolic dysfunction. Macrophages produce TGF-β, and IL-1β, both of which are involved in myocyte hypertrophy.32,33 TGF-β also enhances extracellular matrix synthesis.34 Gene expression of TGF-β, and IL-1β in the left ventricle tended to change with the medication-induced reduction in macrophage infiltration in this study. Thus, macrophage infiltration may play a pivotal role in the development of hypertensive DHF as well as hypertensive systolic heart failure.

TNF-α and MCP-1 have been suggested to contribute to inflammatory changes and pathogenesis of heart failure29,35 and are partly enhanced through renin-angiotensin system.36,37 However, their gene expression did not change with either regimen in this study. Liu et al recently showed that ROS promoted macrophage infiltration,38 and the current data also indicate a close relationship between macrophage infiltration and ROS generation, as assessed with immunohistochemical analysis of HNE. Angiotensin II directly enhances ROS generation.38,39 Therefore, the addition of ARB to ACEI reduced ROS generation, possibly by blocking actions of angiotensin II produced independently of ACE. Superiority of the combination therapy in the reduction of ROS was likely to lead to more suppression of macrophage infiltration and greater benefits for LV structure and diastolic function as compared with the monotherapy.

Study Limitations

There are several limitations in this study. First, we did not examine effects of other doses of temocapril in each of the ACEI and combination groups. Thus, it remains unclear whether a further increase in a dose of temocapril in the ACEI group provides similar effects as compared with the combination therapy. Administration of temocapril at 0.2 mg/kg per day significantly shortened Tau with normalization of Ser16 phosphorylated phospholamban level and effectively prevented hemodynamic deterioration in the same DHF model when initiated at an earlier stage (age 13 weeks) with LV relaxation abnormality and hypertrophy but not with enhanced collagen accumulation or myocardial stiffening.12 In this study, temocapril was administered at the same dose in the combination group and at the doubled dose (0.4 mg/kg per day) in the ACEI group. Thus, the current results indicate at least that benefits of ACEI would be less expected at a later administration, and that the additive effects of ARB might not be mimicked by doubling a dose of ACEI. Second, we did not study effects of longer therapy with either regimen; thus, it remains unclear whether these therapies prevented or delayed the progression of the structural and functional abnormalities in the DHF models. Third, we only have data for age 20 weeks, and detailed temporary changes in LV structure and function for 3 weeks of the medications were not assessed. To address these limitations, further studies are necessary.

Perspectives

The addition of ARB to ACEI achieved more benefits as compared with the monotherapy with ACEI in hypertensive DHF when initiated at the advanced stage with progressive LV hypertrophy and fibrosis, relaxation abnormality, and myocardial stiffening. The additive benefits consist of not only inhibition but also reversal of the structural and functional alterations, at least partly, through prominent suppression of ROS generation and inflammatory changes without effects on expression of MCP-1 and TNF-α.

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