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Conclusions: Surgical treatment for CHF as an interdisciplinary one yields better outcome than before, but there is a room for improvements. Regenerative medicine and/or LV unloading in addition to the surgery may improve the results furthermore in the near future.

PD1-5

Application of Left Ventricular Assists Systems and Heart Transplantation as an Option for Profound Heart Failure Patients

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Objective: We discussed our experience of heart transplant (HTx) candidates with or without left ventricular assist systems (LVAS).
Patients: 64 were male and 26 were female and mean age was 35 years old. 77 were suffered from DCM, 3 from dHCM, and 10 from ICM. Results: 64% of patients were applied several types of LVAS. Mean supporting duration was 473 days and 55% were supported over 1 year. Nineteen LVAS patients were transplanted (11 at NCVC, 8 in USA) after 522 days supports. Five were weaned from LVAS after 173 days supports, and all of them are doing well up to 6.3 years. 22 were died on LVAS after 527 days supports. The other 11 are on going for 424 days. Thirteen patients were received HTx at our center including 11 LVAS patients. Mean waiting time was 579 days. Modified bicaval method was used in 11 and all patients showed good cardiac performance. For basic immunosuppression, triple therapy consisting of cyclospoline-A, mycophenolate mofetil and steroid was selected. Only one died 4.2 years after HTx due to infection. The others were doing well up to 6 years. Conclusion: LVAS and HTx were suitable options for profound heart failure. Further efforts should be done for establishment of HTx program in Japan and introduction of portable implantable LVAS.

PD2-1 *Keynote Lecture*

Diastolic Heart Failure: Diagnosis and Management

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Diastolic heart failure is often diagnosed when symptoms and signs of heart failure occur in the presence of a preserved left ventricular ejection function (PLVEF, normal ejection fraction/normal end-diastolic volume) at rest. However, as these conditions are only equal to some extent, we suggest to use the actual diagnosed cardiac dysfunction. Predominant diastolic dysfunction is relatively uncommon in younger patients but increases in importance in the elderly. PLVEF is more common in women, in whom systolic hypertension and myocardial hypertrophy with fibrosis are contributors to cardiac dysfunction (1,2).
Diagnostic criteria of diastolic dysfunction: The recently published Guidelines on the diagnosis and treatment of chronic heart failure recognise that the following criteria (3):
The three filling patterns "impaired relaxation", " pseudonormalised filling" and "restrictive filling" represent mild, moderate and severe diastolic dysfunction, respectively (4). Thus, by using the combined assessment of transmitral blood flow velocities and mitral annular velocities, it becomes possible to perform staging of diastolic dysfunction during a routine echocardiographic examination. We still lack prospective outcome studies that investigate if assessment of diastolic function by these criteria may improve management of heart failure patients.
Management of heart failure because of PLVEF: There is still little evidence from clinical trials or observational studies on how to treat PLVEF. Further, much debate prevails about the prevalence of heart failure that is due to pure diastolic dysfunction. Although recent epidemiological studies suggest that in the elderly the percentage of patients hospitalised with heart failure-like symptoms and PLVEF may be as high as 35–45%, there is uncertainty about the prevalence of diastolic dysfunction in patients with heart failure symptoms and a normal systolic function in the community. Causes of heart failure because of diastolic dysfunction include myocardial ischaemia, hypertension, myocardial hypertrophy and myocardial/pericardial constriction. These causes should be identified and treated appropriately.
Precipitating factors should be identified and corrected, in particular tachyarrhythmias should be prevented and sinus rhythm restored whenever possible. Rate control is important. Treatment approach is similar to patients without heart failure.
Pharmacological therapy of PLVEF or diastolic dysfunction: The recommendations provided below are largely speculative in that limited data exist in patients with PLVEF or diastolic dysfunction; the reason for the sparsity of data is that patients are excluded from nearly all large controlled trials in heart failure.
Presently, we do not have clear evidence that patients with primary diastolic heart failure benefit from any specific drug regimen. Some evidence is available indicating that patients with heart failure and preserved LVEF benefit from digoxin in the DIG study in a composite of death or hospitalisations for heart failure. Inhibition of the renin-angiotensin system with candesartan in CHARM Preserved (5) reduced cardiovascular mortality or hospitalisations for heart failure slightly and heart failure hospitalisations significantly; mortality, on the other hand, was not influenced. In these studies, however, there was no objective measure of diastolic function and, by consequence, do not permit any conclusion about treatment of diastolic function in general. Because heart failure is most often due to coronary artery disease and/or hypertension, it is most logical to search for these conditions by appropriate tests and then to treat the patients according to general principles for managing these disorders.

1. ACE inhibitors may have long-term effects through their anti-hypertensive effects and regression of hypertrophy and fibrosis.
2. Diuretics may be necessary when episodes with fluid overload are present, but should be used cautiously so as not to lower preload excessively and thereby reduce stroke volume and cardiac output.
3. Beta-blockade could be instituted to lower heart rate and increase the diastolic period.
4. Verapamil-type calcium antagonists may be used for the same reason.
5. A high dose of an ARB may reduce hospitalisations (5).

In general, the treatment of PLVEF/diastolic dysfunction remains difficult and often unsatisfactory. One of the main problems is that isolated diastolic dysfunction may be rare, the condition often occurring in conjunction with some degree of systolic dysfunction. As conditions under which PLVEF/diastolic dysfunction occur vary between patients and no controlled data from studies exist, straightforward therapeutic algorithms are not easy to provide for the individual.

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Original Article

Abnormal Glucose Tolerance Contributes to the Progression of Chronic Heart Failure in Patients with Dilated Cardiomyopathy

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Since 1) dilated cardiomyopathy (DCM) causes chronic heart failure (CHF), and 2) augmentation of neurohumoral factors such as angiotensin II impairs glucose metabolism, we examined the rate of abnormal glucose metabolism in patients having both DCM and CHF and whether correction of the impairment of glucose metabolism would improve the pathophysiology of CHF in DCM patients. A 75-g oral glucose tolerance test (OGTT) was performed in 56 patients with DCM-induced CHF and 168 age- and sex-matched control subjects. Among the CHF patients, 26.8% and 50.0% suffered from diabetes mellitus (DM) and impaired glucose tolerance (IGT), respectively, showing that abnormal glucose tolerance was more prevalent in DCM patients than in the control subjects (7.7% and 14.3%, respectively). In the patients with DCM-induced CHF, a correlation was observed between the brain natriuretic peptide (BNP) levels and the difference between the plasma glucose levels at the time of fasting and at 2 h of OGTT. Since neither DM nor IGT are thought to cause DCM, the abnormalities of glucose metabolism may be attributed to the progression of CHF. Furthermore, we tested whether correction of the abnormal glucose tolerance using voglibose (an α-glucosidase inhibitor) would improve the severity of CHF in another group of 30 patients with DCM-induced CHF and IGT. The patients treated with voglibose for 24 weeks showed decreases in left ventricular dimension, NYHA functional classification values, and plasma BNP levels, and an improvement in cardiac function. In conclusion, abnormal glucose tolerance was more prevalent among patients with DCM-induced CHF than controls, and the correction of IGT improved the pathophysiology of CHF. (*Hypertens Res* 2006; 29: 775–782)

***Key Words*: heart failure, diabetes mellitus, impaired glucose tolerance, voglibose**

Introduction

The regimens for the treatment of patients with chronic heart failure (CHF) include angiotensin-converting enzyme (ACE) inhibitors, β-adrenergic receptor blockers, digitalis and diuretics (*1*). However, despite these medical therapies, since CHF remains one of the major causes of death or hospitalization worldwide, we need to seek a new strategy to treat CHF. CHF is characterized by impaired cardiac performance, inflammation, and neurohormonal imbalance (*2*). Indeed, increases in catecholamine, cytokine, and angiotensin II levels are thought to play important roles in the pathophysiology of CHF (*3–5*). On the other hand, catecholamines and angiotensin II have both been shown to contribute to abnormal glucose tolerance, and either transient high glucose exposure or

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Table 1. Clinical Characteristics of the Control Subjects and DCM Patients

	Control	DCM	Significance
Subjects	168	56	
Age (years old)	55.2±1.1	55.2±1.2	n.s.
Sex (%male (male, female))	76.8 (129, 39)	76.8 (43, 13)	n.s.
Body weight (kg)	56.9±1.0	58.6±1.2	n.s.
Plasma BNP levels (pg/dl)	—	367.1±121.9	
%FS (%)	—	21.4±1.7	
LVDd (mm)	—	58.7±2.1	
NYHA IIs/IIm/III	—	12/29/15	
Diabetes mellitus (%)	7.7	26.8	
IGT (%)	14.3	50.0	$p<0.05$
Normal glucose metabolism (%)	78.0	23.2	
Heart rate (bpm)	71.9±0.45	72.0±0.42	n.s.
Systolic BP (mmHg)	129±0.95	128±0.89	n.s.
Diastolic BP (mmHg)	76.2±0.53	76.0±0.50	n.s.
Total cholesterol levels (mg/dl)	206±1.60	207±2.52	n.s.
Triglicclide levels (mg/dl)	120±3.30	125±6.50	n.s.
Uric acid levels (mg/dl)	5.50±0.16	5.56±0.10	n.s.
Smoking (%)	26.3	22.4	n.s.
Concomitant drugs (%)			
Digoxin		60	
Diuretics		89	
β-Blockers		70	
Angiotensin converting enzyme inhibitors		75	
Angiotensin receptor blockers		12	
Steroids		0	

DCM, dilated cardiomyopathy; BNP, brain natriuretic peptide; FS, fractional shortening; LVDd, left ventricle end-diastolic dimension; NYHA, New York Heart Association; IGT, impaired glucose tolerance; BP, blood pressure. The number in the table is the number of the subjects, mean±SEM or the percentile of the subjects.

decreased insulin sensitivity can result in cellular injury *via* the generation of oxidative stress and provocation of myocardial apoptosis (*6–11*). There are several lines of evidence showing that both high blood glucose level and insulin resistance are major risk factors for CHF (*12–18*), suggesting that either 1) the impairment of glucose metabolism is the primary cause of CHF (*19, 20*) or 2) an impairment of glucose metabolism newly developed during the progression of CHF contributes to the worsening of CHF (*20–22*). In a clinical setting, it is difficult to determine whether or not the impairment of glucose metabolism follows the occurrence of CHF and contributes to the progression of CHF. One strategy for resolving this question would be to investigate the prevalence of the impairment of glucose metabolism in patients with CHF caused by dilated cardiomyopathy (DCM), because neither diabetes mellitus (DM) nor impaired glucose tolerance (IGT) is believed to cause primary DCM. If the prevalence of DM or IGT is higher in patients with DCM-induced CHF, we suggest the abnormalities of glucose metabolism may occur along with the progression of CHF

To test this hypothesis, we examined the prevalence of abnormalities of glucose metabolism revealed by not only fasting glucose levels but also an oral glucose tolerance test (OGTT) in patients with DCM-induced CHF. Furthermore, we tested whether administration of an α-glucosidase inhibitor (αGI) for 24 weeks would improve the severity of CHF in 30 patients with DCM-induced CHF and IGT.

Methods

Protocol I: The Prevalence of Impairment of Glucose Metabolism in DCM Patients with CHF and Normal Control Subjects

We studied 56 DCM patients with symptomatic CHF between January 2004 and January 2005. The criteria for enrollment in this study were 1) clinical diagnosis of DCM, 2) clinical evidence of CHF despite conventional therapy as quantified by a New York Heart Association (NYHA) functional classification of II to III, and 3) a left ventricular fractional shortening (FS) below 30%, as assessed by two-dimensional echocardiography. There were 43 men and 13 women with a mean age of 55.2 years. Using coronary angiography, a ventriculogram, myocardial biopsy or echocardiography, all patients were

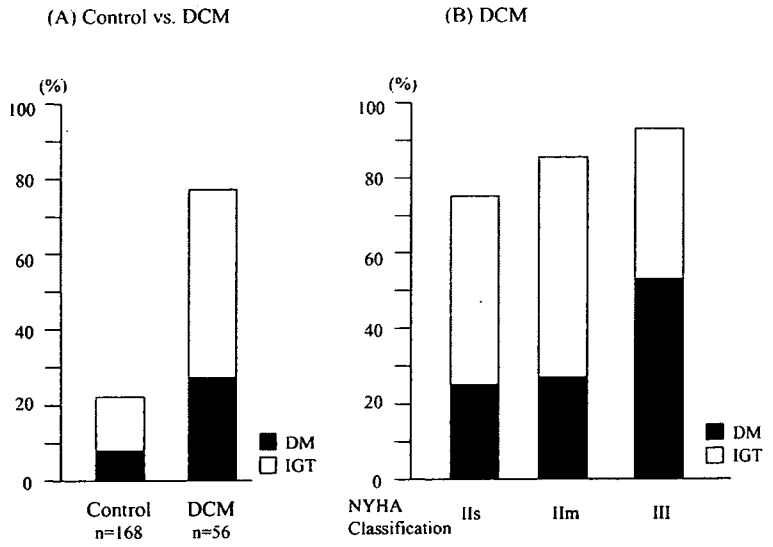

Fig. 1. *Prevalence of either impaired glucose tolerance (IGT) or diabetes mellitus (DM) in patients with DCM-induced CHF or healthy control subjects. A: The incidence of either DM or IGT in the CHF group was higher ($p<0.001$) than in the control group. B: The DCM patients were classified according to the New York Heart Association (NYHA) classification.*

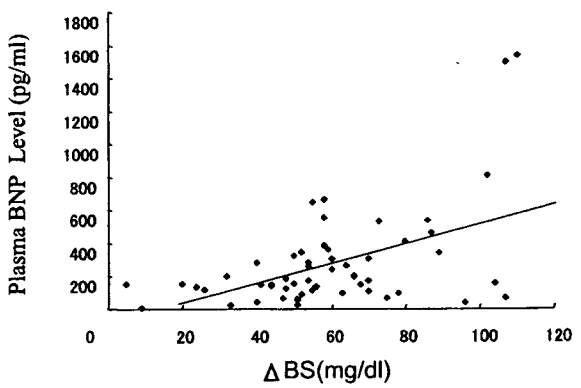

Fig. 2. *The relationship between the BNP level and the change in glucose level between fasting and 2 h after the 75-g OGTT (ΔBS). These two parameters were linked to each other (the plasma BNP level [pg/ml] = −117.0 + 6.516 ΔBS [mg/dl], $p<0.001$, $r^2=0.259$).*

diagnosed as having DCM. Exclusion criteria were 1) chronic obstructive pulmonary disease, 2) either history or evidence of ischemic heart disease, 3) cerebrovascular disease, 4) peripheral vascular disease, 5) cancer, 6) estrogen replacement therapy, 7) insulin-dependent DM, 8) any disease that may secondarily cause DM, 9) the use of drugs that can induce DM, such as steroids, and 10) pregnancy. The severity of CHF was assessed by the plasma brain natriuretic peptide (BNP) levels, the echocardiographic data and the NYHA classification. BNP levels were measured using a specific immunoradiometric assay. Informed consent was obtained from each patient before participation in this study in accordance with institutionally approved protocols. As a control, we enrolled 168 age- and sex-matched subjects who had a community health examination at Suita City. Three control subjects were for each patient. Subjects with a past history of heart disease were excluded from the analysis. The fasting plasma glucose levels and the plasma glucose levels 120 min after the 75-g OGTT test were examined in all of the patients except those who had already been diagnosed with DM before entry into the study.

Protocol II: A Prospective Study to Test the Effect of Voglibose on Plasma BNP Levels and the Parameters Obtained from Echocardiography in Patients with DCM-Induced CHF and IGT

We studied 30 DCM patients with symptomatic CHF at our institute. The criteria for enrollment in this study were 1) clinical diagnosis of DCM, 2) clinical evidence of CHF despite conventional therapy, 3) the presence of abnormalities of IGT and 4) low left ventricular FS (below 30 %) as assessed by two-dimensional echocardiography. All the patients had NYHA functional classifications of II to III. DCM was diagnosed as in Protocol I. There were 17 men and 13 women with a mean age of 56 years. Exclusion criteria included chronic obstructive pulmonary disease, pregnancy, and severe liver disease, which was defined as a hepatic enzyme level more than twice the upper limit of normal. All patients were treated by optimal and stable doses of β-blockers and ACE inhibitors for at least 3 months before screening echocardiography and

Table 2. Baseline Clinical Characteristics of the CHF Patients Treated with or without Voglibose

	Control group	Voglibose group	Significance
Subjects	15	15	
Age (years old)	57.1±3.2	54.9±3.3	n.s.
Sex (male:female)	8:7	9:6	n.s.
LVDd (mm)	67.8±3.1	65.5±2.7	n.s.
FS (%)	16.3±2.1	15.8±1.7	n.s.
Heart rate (bpm)	79±2.3	84±3.3	n.s.
Systolic BP (mmHg)	112±3.3	107±3.2	n.s.
Diastolic BP (mmHg)	67.2±2.2	64.3±3.4	n.s.
Pre NYHA class (II/III)	4/11	6/9	n.s.

CHF, chronic heart failure; other abbreviations are the same as in Table 1. The number in the table is either number of the subjects or mean±SEM.

randomization. Patients were randomly divided into the groups with and without voglibose ($n=15$ for the voglibose group, and $n=15$ for the control group). The dose of voglibose was 0.3 mg just before each meal, and there were no patients who discontinued the intake of either voglibose or drugs for CHF.

The primary endpoints were a change in the NYHA functional class and a change in the plasma BNP levels. Additional analyses were performed using the echocardiogram to obtain the changes in left ventricular or atrial dimensions. A randomization was performed according to a randomization list generated by computers at the Clinical Study Support Center of Japan (Suita, Japan).

Measurements

NYHA Classification

Functional class was evaluated by certified cardiologists, according to the NYHA classification, after the clinical examinations. Heart failure was defined as NYHA class I–III at baseline. We did not enroll the patients with NYHA IV because catecholamines are administered in such patients and may modulate the glucose metabolism.

The NYHA classification of each patient was estimated by 3 independent cardiologists who did not have knowledge of the patients. If the 3 estimations did not agree, we used the median of the 3 values.

IGT and DM

In 1997, the American Diabetes Association adopted new criteria for diabetes (*23*, *24*). We followed these criteria in the present study: patients with a single fasting blood glucose level of more than 125 mg/dl were considered to have DM. In the other subjects, we performed a 75-g OGTT, and IGT was considered to be present in patients whose glucose levels at 120 min after intake of 75 g of glucose were between 140–199 mg/dl, and patients whose glucose levels were more than 199 mg/dl were also classified as having DM.

Echocardiography

Echocardiograms were performed and checked by cardiologists who had no information about the patients. Echocardiographic measurements were performed using the guidelines of the American Society of Echocardiography. Left ventricular end-diastolic and end-systolic dimensions (LVDd and LVDs, respectively), and left atrial diameter (LAD) were recorded by M-mode (SSA 260A, SSH 160A [Toshiba, Tokyo, Japan], Sonos 2000 [Hewlett Packard, CA, USA], SSD 870, or SSD 2200 [ALOKA, Tokyo, Japan]). We calculated FS (%) as (LVDd − LVDs) × 100/LVDd. Fifteen technicians trained in cardiac echocardiography randomly obtained echocardiograms of all of the patients in a single echo-laboratory at our institute, and these results were verified by two cardiologists.

Plasma BNP Measurement

Blood was sampled from each patient in the sitting position in a syringe containing both EDTA (1 mg/dl) and aprotinin (103 kIU/ml). Serum was separated within 6 h and the samples were stored at −20°C until the measurements. The concentration of BNP was measured within 1 week after the plasma sampling by an immunoradiometric assay (IRMA) method (Shionoria BNP test at the SRL Laboratory, Tokyo, Japan). This test is a one-step immunoradiometric assay that uses two different monoclonal antibodies that recognize the C-terminal structure and the disulfide bond–mediated ring structure of BNP 32, respectively.

Statistical Analysis

Baseline characteristics were compared by Fisher's exact, χ^2 test or Cochran-Mantel-Haenszel test for categorical variables. ANOVA was used to test for treatment-group baseline differences for continuous variables. Within-treatment analyses of changes were performed using a Student's t-test, and values of $p<0.05$ were considered to indicate statistical significance (*25*).

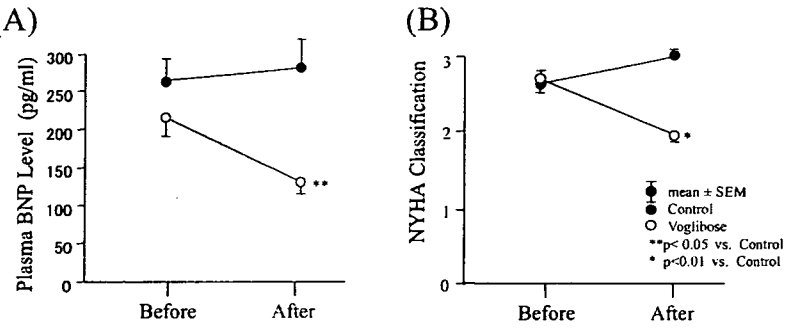

Fig. 3. *Plasma BNP level (A) and NYHA Functional classification (B) before and after the treatment with voglibose. The treatment with voglibose decreased plasma BNP level and NYHA class, but neither plasma BNP level nor NYHA class was changed in the control group.*

Results

Table 1 shows the patients' characteristics in Protocol I. Among the 56 CHF patients with symptomatic DCM, 12 patients (21%) were in NYHA class IIs, 29 patients (52%) in class IIm, and 15 patients (27%) in class III. They were treated with digitalis, diuretics, β-blockers, and/or angiotensin converting enzyme inhibitors. Twenty-six point eight percent and 50.0% of the 56 patients suffered from DM and IGT, respectively (Fig. 1A). Among the 168 control subjects, 7.7% and 14.3% suffered from DM and IGT, respectively (Fig. 1A). The incidences of both DM and IGT in the CHF group were higher ($p<0.001$) than those in the control group. Furthermore, the incidences of both DM and IGT increased as the severity of CHF assessed by NYHA classification progressed (Fig. 1B). There were no significant differences in medication for CHF, especially diuretics or β-blockers, between the patients with and those without either DM or IGT. Diuretics were used in 88% of the patients with either DM or IGT, and in 92% of the patients without either DM or IGT. β-Blockers were used in 70% of the patients with either DM or IGT, and in 69% of the patients without either DM or IGT, indicating that the use of diuretics and the use of β-blockers did not differ between patients with and those without either DM or IGT. Among the CHF patients who received a 75-g OGTT, the BNP levels were correlated with the change in glucose level between fasting and 2 h after the 75-g OGTT (ΔBS) (n= 56, $p<0.001$, Fig. 2). We also investigated 189 CHF patients diagnosed with DCM, hypertensive heart disease and primary valvular disease and obtained the same results (data not shown).

We administered voglibose to the CHF patients due to the patients with IGT and DCM-induced CHF for 24 weeks. Table 2 shows the patients' characteristics at baseline. All patients completed the protocol, and no patients died during the 24-week study. In addition, the doses of voglibose and the other drugs for CHF were not altered during the study. Neither blood pressure nor heart rate differed between the groups with and without voglibose before the treatment (systolic blood pressure [SBP]: 107±3 *vs.* 112±3 mmHg; diastolic blood pressure [DBP]: 64±3 *vs.* 67±2 mmHg; heart rate: 84±3 *vs.* 79±2 bpm, respectively), and there were no significant changes in any of these parameters at 24 weeks after the onset of the study in either the group with or that without voglibose (SBP: 109±3 *vs.* 110±3 mmHg; DBP: 62±2 *vs.* 65±2 mmHg; heart rate: 80±2 *vs.* 82±2 bpm, respectively). The plasma BNP levels and NYHA classification significantly decreased (Fig. 3), and LVDd, LVDs and LAD significantly decreased and FS significantly increased (Fig. 4) in the patients in the voglibose group compared with the control group. This indicates that voglibose ameliorated the severity of CHF.

Discussion

The present study has demonstrated that abnormalities of glucose metabolism are tightly associated with the severity of CHF, and contribute to the deterioration of CHF in patients with DCM. Most importantly, this is the first report to show that correction of the abnormalities of glucose metabolism using αGI in patients with DCM-induced CHF and IGT improved the pathophysiology of CHF.

CHF and Abnormalities of Glucose Metabolism

Several reports have described a relationship between abnormalities of glucose metabolism and the progression of CHF, and have suggested that abnormalities of glucose metabolism contribute to the pathophysiology of CHF (*12–17*). Several investigations have shown that DM, and even relatively mild glucose abnormalities, are strongly associated with cardiovascular morbidity and mortality (*26–28*), indicating that DM is an independent risk factor for CHF (*18–23*). However, the opposite may also be true, as was suggested in the present study, *i.e.*, the fact that both DM and IGT may themselves be

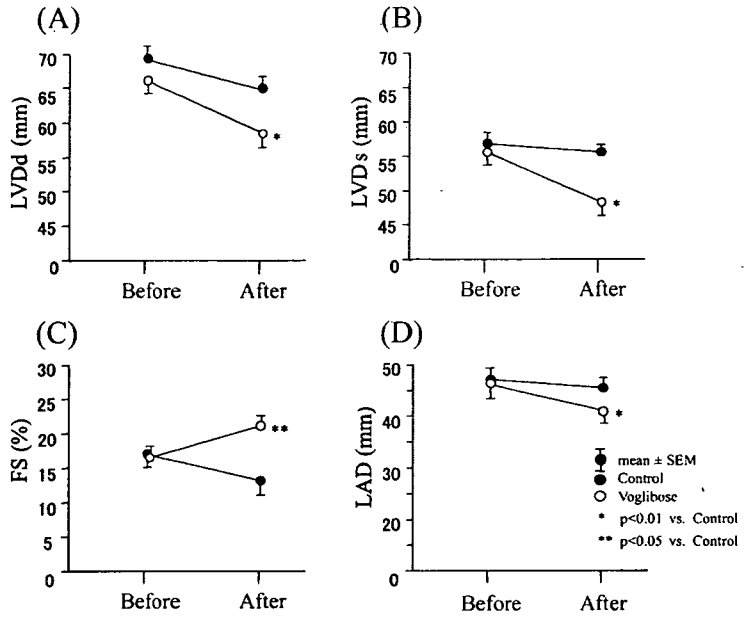

Fig. 4. *Changes in left ventricular end-diastolic volume (LVDd) (A) or end-systolic volume (LVDs) (B), LV fractional shortening (FS) (C) and left atrial diameter (LAD) (D) before and after the treatment for 24 weeks in the voglibose groups. These parameters were not changed in the control group.*

caused by CHF. Since 1) we enrolled patients with DCM-induced CHF, and 2) neither DM nor IGT seems to cause DCM, the abnormalities of glucose metabolism may not necessarily precede the incidence of CHF. Rather, the abnormalities of glucose metabolism may occur along with the progression of CHF, which is a major hypothesis of the present study. There is a report that CHF was associated with the subsequent development of non–insulin-dependent diabetes mellitus (NIDDM) in a group of elderly subjects (*21*), and the present study of the frequent prevalence of abnormalities of glucose metabolism in DCM patients with CHF also supports the present hypothesis. Intriguingly, in addition to the results of Amato *et al.* (*21*), we further observed that IGT is also tightly involved in the pathophysiology of CHF. This is confirmed by the relationship between the severity of either IGT or DM and CHF, and the results of voglibose-induced improvements of CHF shown in the present study.

Possible Mechanisms by Which CHF Causes Abnormal Glucose Tolerance

CHF is known to be an insulin-resistant state that may constitute one of the major risk factors for the development of NIDDM (*13*, *14*, *16*, *20*). Advanced CHF leads to progression of insulin resistance, characterized by fasting and stimulated hyperinsulinemia (*14*), which is a major risk factor for the development of DM (*29*).

There are several possible mechanisms by which CHF may trigger either IGT or DM. First, since either β-blockers or diuretics, which may cause either DM or IGT, are used in CHF patients, these drugs may induce either IGT or DM. However, this was not the case in the present study, because these drugs were equally used in CHF patients with or without abnormal glucose tolerance. Secondly, patients with CHF often show reduced physical activity, which may increase the risk of IGT or DM (*30*). Either insulin resistance or reduced physical activity, or both, may therefore explain the increased risk of abnormal glucose tolerance in patients with CHF. The third possibility is the contribution of free fatty acids (FFA); catecholamines, which are elevated as the pathophysiology of CHF progresses, increase the FFA levels in the adipose tissue, and the elevated FFA levels increase the expression levels of mitochondrial uncoupling proteins and decrease the expression of GLUT4, which promotes the uptake of glucose (*31*). The fourth possibility is that angiotensin II is involved. Angiotensin II, which is elevated in patients with CHF, decreases the insulin sensitivity and impairs the β cells of the pancreas (*32*). The fifth possibility is that BNP, which is also elevated in patients with CHF, played a role in the present results. However, this possibility is less likely because BNP increases adiponectin, which may improve the glucose tolerance. Any one of these factors, or several in combination, may be the cause of the abnormalities of glucose metabolism.

Cellular Mechanisms by Which Abnormal Glucose Abnormality Ameliorates CHF

High glucose exposure, even for a short length of time, produces oxidative stress, and provokes cellular damages such as necrosis or apoptosis (*8–11*). If this occurs in cardiomyocytes, myocardial dysfunction may be worsened by DM or even IGT. Indeed, postprandial hyperglycemia is an indicator of myocardial perfusion defects in DM patients (*33*). Secondly, insulin resistance that causes energy depletion of the myocardium may provoke cardiac dysfunction. Thirdly, the abnormalities of glucose metabolism cause the impairments of endothelial cells attached to cardiomyocytes, and the endothelial dysfunction may be involved in the deterioration of CHF. This is because NO is known to be beneficial for cardiomyocytes as well as smooth muscle cells (*34*), and we have previously reported that depletion of NO causes cardiac hypertrophy or coronary insufficiency *via* activation of angiotensin II followed by the activation of either ERK or P70 S6 kinase (*35*). Indeed, Hirooka *et al.* reported that endothelial function and angiotensin II compete with each other (*36*).

Clinical Impact of the Present Observations

The present study should have an impact on the treatment of CHF. If, in fact, the prevalence of abnormal glucose tolerance is very high among CHF patients worldwide, voglibose may become a novel therapy for the treatment of patients with CHF and abnormal glucose metabolism. Furthermore, if either transient high glucose exposure or decreased insulin sensitivity makes the failing myocardium worse, voglibose may improve the severity of CHF. Further investigations will be needed to examine this point.

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Impact of Blockade of Histamine H_2 Receptors on Chronic Heart Failure Revealed by Retrospective and Prospective Randomized Studies

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OBJECTIVES The goal of this work was to determine whether the blockade of histamine H_2 receptors is beneficial for the pathophysiology of chronic heart failure (CHF).

BACKGROUND Because CHF is one of the major life-threatening diseases, we need to find a novel effective therapy. Intriguingly, our previous study, which predicts the involvement of histamine in CHF, suggests that we should test this hypothesis in patients with CHF.

METHODS We selected 159 patients who received famotidine among symptomatic CHF patients for the retrospective study. We blindly selected age- and gender-matched CHF patients receiving drugs for gastritis other than histamine H_2 receptor blockers as a control group. For the prospective study, 50 symptomatic CHF patients were randomly divided into 2 groups. One group received famotidine of 30 mg/day for 6 months, and the other group received teprenone.

RESULTS In the retrospective study, famotidine of 20 to 40 mg decreased both left ventricular end-diastolic and end-systolic lengths (LVDd and LVDs, respectively) and the plasma B-type natriuretic peptide (BNP) levels (182 ± 21 vs. 259 ± 25 pg/ml, $p < 0.05$) with unaltered fractional shortening (FS). In a randomized, open-label study, compared with teprenone, famotidine of 30 mg prospectively decreased both New York Heart Association functional class ($p < 0.05$) and plasma BNP levels (183 ± 26 pg/ml vs. 285 ± 41 pg/ml, $p < 0.05$); this corresponded to decreasing both LVDd (57 ± 2 mm vs. 64 ± 2 mm, $p < 0.05$) and LVDs (47 ± 2 mm vs. 55 ± 2 mm, $p < 0.05$) with unaltered FS (15 ± 1% vs. 17 ± 1%). The frequency of readmission because of worsening of CHF was lower in the famotidine group (4% and 24%, $p < 0.05$). On the other hand, teprenone had no effects on CHF.

CONCLUSIONS Famotidine improved both cardiac symptoms and ventricular remodeling associated with CHF. Histamine H_2 receptor blockers may have therapeutic benefits for CHF. (J Am Coll Cardiol 2006;48:1378–84) © 2006 by the American College of Cardiology Foundation

Despite current medical therapy for patients with chronic heart failure (CHF) such as angiotensin-converting enzyme (ACE) inhibitors or beta-adrenergic receptor blockers (1), CHF remains one of the major causes of high morbidity and mortality worldwide. Chronic heart failure is characterized by cardiac symptoms, impaired cardiac performance, cardiac

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mechanical stress, and neurohormonal imbalance (2). Indeed, increased levels of catecholamines, cytokines, and angiotensin II are thought to play important roles in the pathophysiology and development of CHF (3,4). Histamine is one of the neurohormonal factors that provoke various cellular functions via stimulation of histamine H_1-H_3 receptors (5,6). Specifically, because histamine H_2 receptors are known to be located in gastric cells and enhance the production of acids that cause gastric ulcers, the blocker of histamine H_2 receptors is developed as the drug for the treatment of gastric ulcers (7). Interestingly, we have previously predicted that histamine H_2 receptor blockers may be cardioprotective in patients with CHF using the data mining technique (8). The histamine H_2 receptor is also located in the cardiomyocytes, and this receptor is coupled to Gs protein as well as is the beta receptor (9–13). Indeed, it is reported that: 1) histamine provokes positive inotropic effects (11,14); and 2) the blocker of histamine H_2 receptors decreases cardiac output (14). The important roles of mast cells and released histamine are also accepted in the cardiovascular system (15).

We tested the hypothesis that the blockade of histamine H_2 receptors by famotidine is beneficial for the pathophys-

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Abbreviations and Acronyms

ACE = angiotensin-converting enzyme
AMP = adenosine monophosphate
BNP = brain natriuretic peptide
CHF = chronic heart failure
FS = fractional shortening
GERD = gastroesophageal reflux disease
LVDd = left ventricular end-diastolic volume
LVDs = left ventricular end-systolic volume
NYHA = New York Heart Association

iology of CHF in retrospective and prospective randomized studies.

METHODS

This study was approved by the ethical committee of National Cardiovascular Center. Informed consent was obtained from all patients before participation in this study in accordance with institutional approved protocols.

Study population and protocols. THE RETROSPECTIVE STUDY. A total of 1,104 consecutive subjects who were admitted to our hospital for treatment of CHF between January 2002 and April 2004 were candidates for this study. The criteria for enrollment in this study were: 1) clinical evidence of heart failure despite the conventional therapy; and 2) left ventricular fractional shortening (FS) below 30%, as assessed by 2-dimensional echocardiography. All the patients had New York Heart Association (NYHA) functional classifications of II to III, but were stable for 2 months after their discharge. Among these patients, we selected the patients who received famotidine of 20 to 40 mg (n = 159, the famotidine group). In the control group, the patients were selected so as to be matched for age, gender, and the cause of CHF (n = 159). We randomly selected age-, gender-, and cause-matched patients for the other drug of non-histamine H_2 blocker for gastritis (n = 159). Among the 159 patients in each group, the number of patients who suffered from dilated cardiomyopathy, hypertensive heart disease, ischemic cardiomyopathy, and valvular heart disease were 71, 11, 39, and 38, respectively. Clinical parameters of the plasma brain natriuretic peptide (BNP) levels and echocardiography were obtained. We estimated the NYHA functional classification of each patient by 3 independent cardiologists who were blinded to the medical treatment of CHF. If the estimations of all 3 doctors did not agree, we decided to take the median among 3 values of NYHA functional classification.

The prospective studies: the effects of famotidine. We studied 50 patients with symptomatic CHF and gastroesophageal reflux disease (GERD) in our institute. Gastroesophageal reflux disease was diagnosed by questionnaires reported previously (16). The criteria for enrollment in this study were clinical evidence of heart failure despite the conventional therapy and a left ventricular FS below 30%, as assessed by 2-dimensional echocardiography, and existence of GERD. All the patients had NYHA functional classifications of II to III. There were 32 men and 18 women with a mean age of 65 years. The number of patients diagnosed as CHF because of dilated cardiomyopathy, hypertensive heart disease, ischemic cardiomyopathy, and valvular heart disease were 17, 2, 4, and 2 in each group, respectively. Exclusion criteria included chronic obstructive pulmonary disease, pregnancy, and severe liver disease as defined by having hepatic enzymes >2 times the upper limit of normal values. All patients were treated by optimal and stable doses of beta-blockers and ACE inhibitors for at least 3 months before screening echocardiography and randomization. We did not change the doses of these drugs after the enrollment. Patients were randomly divided into 2 treatment groups: famotidine (n = 25, the famotidine group) and teprenone (n = 25, the control group). The doses of famotidine and teprenone were 30 and 150 mg per day, respectively, and there were no patients who discontinued the intake of either famotidine or teprenone, and drugs for CHF.

In the current study, we tested the hypothesis that famotidine, the histamine H_2 receptor blocker, may have therapeutic benefits for CHF in the clinical settings. The primary end point is to assess the changes in NYHA functional class and the plasma BNP levels from the baseline to 24 weeks. We estimated the NYHA functional classification of each patient by 3 independent cardiologists who were blinded to the treatment assignment of famotidine. If the estimations of all 3 doctors did not agree, we decided to take the median among 3 values of NYHA functional classification. Additional analyses were done using the echocardiogram to obtain the changes in left ventricular or atrial volume, and the pressure differences across the tricuspid valve from baseline to 24 weeks. Furthermore, the frequency of readmission because of worsening of CHF within 24 weeks was investigated.

Estimating from retrospective study results showing that the reduction of the plasma BNP levels was about 30%, 25 patients were required for each study group. A randomization was performed according to a computer generated randomization list by central telephone call or fax to Clinical Study Support Center Japan (Suita Osaka, Japan).

Effects of teprenone. There is a possibility that teprenone has deleterious effects on the pathophysiology of CHF, and if this were the case, famotidine would appear to be beneficial, when famotidine has no cardioprotective effects. To examine this possibility, we administered teprenone to 10 patients with CHF for 24 weeks, and compared 10 CHF patients without the teprenone treatment. The criteria for the enrollment, evaluated parameters, and the evaluation procedure were the same as in the study of famotidine described earlier in the text.

Analysis of parameters for CHF. Blood samples were collected in test tubes containing ethylenediaminetetraacetic acid at baseline and after 24 weeks of the treatment. The plasma was separated from blood cells by centrifugation and frozen at −80°C. Plasma concentrations of BNP were

measured using a specific immunoradiometric assay (17). The personnel performing these assays were blinded to the patients' treatment assignments.

M-mode echocardiography was performed with 2-dimensional monitoring using a Sono layer phased-array sector scanner (SONOS 5500, Hewlett Packard, Palo Alto, California) before and after 24 weeks of the treatment with famotidine or teprenone (18). All echocardiograms were read by the same physician, at baseline and after 24 weeks of the treatment, who was blinded to patients' treatment, assignment, and time point.

Statistical analysis. Data are presented as mean ± SEM. Statistical analysis was performed using paired or unpaired *t* test for numerical values, and either chi-square tests or Wilcoxon signed rank test for categorical values. B-type natriuretic peptide levels were logarithmically transformed to perform the statistical analysis. Furthermore, we used two-way repeated-measures analysis of variance when we compared the changes of each parameter in 2 groups. The chi-square tests were also performed to test the differences of the incidence of the readmission. All statistical analyses were performed using Stat View version 5.0 for Windows (SAS Institute, Cary, North Carolina) and SPSS 10.0.5J software (SPSS Inc., Chicago, Illinois).

Table 1. Clinical Parameters of CHF With or Without Famotidine

	Control Group (n = 159)	Famotidine Group (n = 159)
Age (yrs)	66 ± 1	66 ± 1
M/F gender (%)	97/62 (61/39)	97/62 (61/39)
Hypertension (%)	11 (7)	11 (7)
Duration of CHF (yrs)	8.7 ± 0.7	8.5 ± 0.8
Systolic blood pressure (mm Hg)	112 ± 9	105 ± 8*
Diastolic blood pressure (mm Hg)	67 ± 4	62 ± 5*
Heart rate (beats/min)	73 ± 5	66 ± 5*
Fractional shortening (%)	24 ± 1	23 ± 1
LV diastolic diameter (mm)	58 ± 2	54 ± 1*
LV systolic diameter (mm)	44 ± 1	41 ± 1*
LA diameter (mm)	40 ± 3	39 ± 3
Pressure across tricuspid valve (mm Hg)	30 ± 2	28 ± 2
Plasma BNP levels (pg/ml)	259 ± 25	182 ± 21*
NYHA functional class: II/III (%)	75/84 (47/53)	97/62 (61/39)*
Concomitant drugs, n (%)		
Digoxin	126 (80)	134 (84)
Diuretics except spironolactone	140 (88)	137 (86)
Nitrates	80 (25)	32 (20)
Beta-blockers	143 (90)	137 (86)
ACE inhibitors	127 (80)	121 (76)
ARB	118 (20)	38 (24)
Spironolactone	25 (20)	25 (20)

Values are either numbers of each group, range, or mean ± SEM. *$p < 0.05$ vs. the control group.

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; BNP = brain natriuretic peptide; CHF = chronic heart failure; LA = left atrium; LV = left ventricular; NYHA = New York Heart Association; Fractional shortening (%) = (left ventricle end-diastolic diameter − left ventricle end-systolic diameter)/left ventricle end-diastolic diameter.

Table 2. Baseline Characteristics of the Study Population

	Teprenone Group (n = 25)	Famotidine Group (n = 25)
Age (yrs)	65 ± 2	65 ± 2
M/F gender (%)	16/9 (64/36)	16/9 (64/36)
NYHA functional class: II/III (%)	8/17 (32/68)	10/15 (40/60)
Hypertension (%)	4 (16)	6 (24)
Duration of CHF (yrs)	11.2 ± 1.7	13.2 ± 2.3
Systolic blood pressure (mm Hg)	113 ± 3	112 ± 3
Diastolic blood pressure (mm Hg)	68 ± 3	67 ± 2
Heart rate (beats/min)	83 ± 3	83 ± 2
Fractional shortening (%)	15 ± 1	15 ± 1
LV diastolic diameter (mm)	65 ± 2	64 ± 2
LV systolic diameter (mm)	55 ± 2	55 ± 2
LA diameter (mm)	43 ± 2	42 ± 2
Pressure across tricuspid valve (mm Hg)	33 ± 3	36 ± 3
Plasma BNP levels (pg/ml)	268 ± 28	286 ± 41
Concomitant drugs, n (%)		
Digoxin	24 (96)	22 (84)
Diuretics except spironolactone	25 (100)	25 (100)
Nitrates	7 (28)	4 (16)
Beta-blockers	25 (100)	25 (100)
ACE inhibitors	23 (92)	21 (84)
ARB	2 (8)	4 (16)
Spironolactone	10 (40)	8 (32)

Values are either numbers of each group, range, or mean ± SEM. Abbreviations as in Table 1.

RESULTS

After age and gender matching, as shown in Table 1, the gender ratio and the average age of the 2 groups were similar. There were no significant differences of the variety of medical treatment drugs between the 2 groups. Blood pressure, heart rate, NYHA functional class, the plasma BNP levels, and left ventricular dimensions were smaller in the famotidine group compared with the control group. There were no differences between FS in the 2 groups. This result suggests that famotidine may be beneficial for pathophysiology of CHF.

As for the prospective randomized famotidine treatment protocol, medications were well tolerated over the 24-week period. The participants were recruited from September 2004 to October 2004. Participants attended clinic visits at the time of randomization (baseline) and at 4- to 8-week intervals for 24 weeks. All patients completed the protocol (50 of 50). No patients died during the 24-week study. In addition, the doses of beta-blockers, ACE inhibitors, and diuretics were not altered during the course of the entire study.

There were no differences in age, gender, or concurrent medications between the control and famotidine groups (Table 2). Blood pressure and heart rate were not different between the groups with and without famotidine before the treatment. Famotidine administration slightly decreased blood pressure (systolic and diastolic blood pressure 107 ± 3 mm Hg vs. 112 ± 3 mm Hg, $p < 0.01$ and 60 ± 3 mm Hg vs. 67 ± 2 mm Hg, $p < 0.05$) and heart rate (79 ± 2

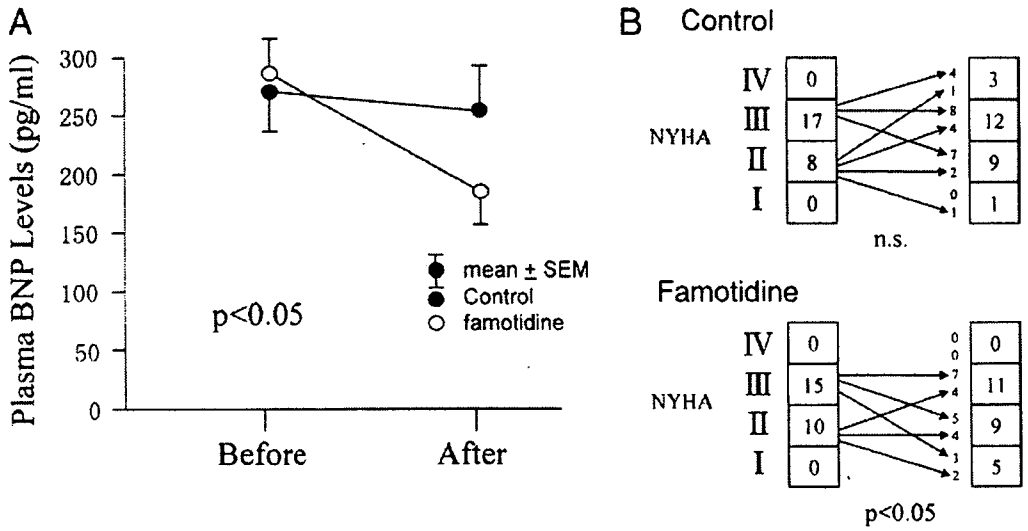

Figure 1. Changes in the plasma B-type natriuretic peptide (BNP) levels **(A)** and New York Heart Association (NYHA) functional classification **(B)** before and after the treatment with (the famotidine group) or without famotidine (the control group). The plasma BNP levels are statistically analyzed after the log transformation. The p values are obtained using 2-way repeated-measures analysis of variance **(A)** or Wilcoxon signed rank test **(B)**.

min^{-1} vs. 83 ± 2 min^{-1}, $p < 0.05$), whereas the control group did not exhibit changes in either blood pressure (systolic and diastolic blood pressure 113 ± 3 mm Hg vs. 113 ± 3 mm Hg and 68 ± 3 mm Hg vs. 68 ± 3 mm Hg) or heart rate (81 ± 3 min^{-1} vs. 83 ± 3 min^{-1}) before and 24 weeks after the treatment. The patients who received famotidine demonstrated improved functional capacity assessed by the plasma BNP levels and NYHA functional class (Fig. 1). Plasma BNP level and NYHA functional class were unchanged after 24 weeks in the group without famotidine. The functional improvement in the famotidine group was associated with improved cardiac performance. Compared with the group without famotidine, the patients treated with famotidine had lower left ventricular end-diastolic volume (LVDd) and left ventricular end-systolic volume (LVDs) while keeping FS unchanged (Fig. 2). The

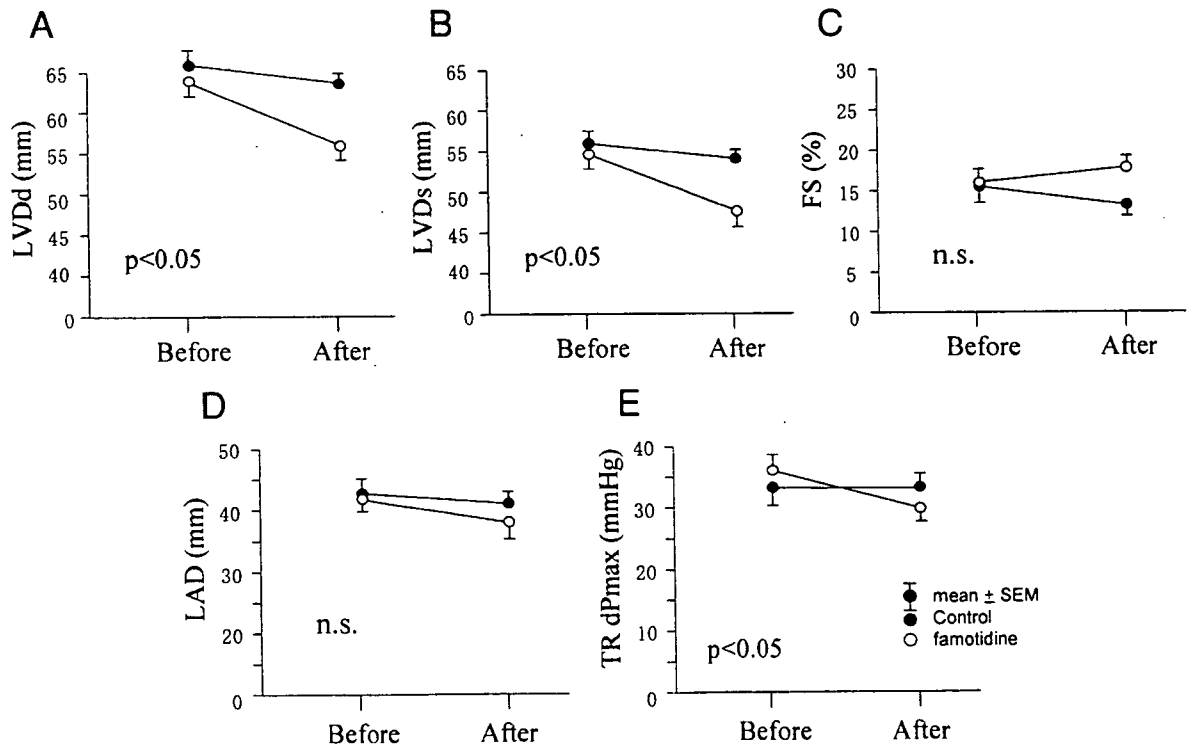

Figure 2. Changes in left ventricular (LV) end-diastolic volume (LVDd) **(A)** or end-systolic volume (LVDs) **(B)**, LV fractional shortening (FS) **(C)**, left atrial diameter (LAD) **(D)**, and the pressure differences across the tricuspid valve (TR dPmax) **(E)** before and after 24 weeks of treatment in the control and famotidine groups. The p values are tested using 2-way repeated-measures analysis of variance.

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Table 3. Baseline Characteristics of the Study Population

	Teprenone Group (n = 10)	No Treatment Group (n = 10)
Age (yrs)	67 ± 4	68 ± 3
M/F gender (%)	7/3 (70/30)	7/3 (70/30)
NYHA functional class: II/III (%)	2/8 (20/80)	2/8 (20/80)
Hypertension (%)	2 (20)	2 (20)
Duration of CHF (yrs)	12.1 ± 2.8	12.0 ± 1.3
Systolic blood pressure (mm Hg)	109 ± 5	114 ± 4
Diastolic blood pressure (mm Hg)	64 ± 4	65 ± 32
Heart rate (beats/min)	75 ± 4	75 ± 2
Fractional shortening (%)	18 ± 3	18 ± 1
LV diastolic diameter (mm)	66 ± 4	65 ± 1
LV systolic diameter (mm)	53 ± 3	54 ± 1
LA diameter (mm)	45 ± 2	45 ± 2
Pressure across tricuspid valve (mm Hg)	36 ± 3	34 ± 2
Plasma BNP levels (pg/ml)	246 ± 48	248 ± 26
Concomitant drugs, n (%)		
Digoxin	10 (100)	10 (100)
Diuretics except spironolactone	10 (100)	10 (100)
Nitrates	2 (20)	1 (10)
Beta-blockers	10 (100)	10 (100)
ACE inhibitors	9 (90)	7 (70)
ARB	0 (0)	3 (30)
Spironolactone	5 (50)	5 (50)

Values are either numbers of each group, range, or mean ± SEM.
Abbreviations as in Table 1.

frequency of readmission because of worsening of CHF was lower in the famotidine group compared with the control group (1 [4%] and 6 [24%], difference [95% confidence interval] 20% [2 to 38], $p < 0.05$).

As for the prospective randomized teprenone treatment protocol, medications were well tolerated over the 24-week period. The participants were recruited from September 2004 to October 2004. Participants attended clinic visits at the time of randomization (baseline) and at 4- to 8-week intervals for 24 weeks. All patients completed the protocol (20 of 20). No patients died during the 24-week study. In addition, the doses of beta-blockers, ACE inhibitors, and diuretics were not altered during the course of entire study. There were no differences in age, gender, or concurrent medications between the control and famotidine groups (Table 3). We found that teprenone does not affect the severity of CHF (the plasma BNP levels: 246 ± 48 pg/ml vs. 234 ± 49 pg/ml, LVDd: 65.8 ± 3.6 mm vs. 65.6 ± 3.7 mm, LVDs: 53.2 ± 2.9 mm vs. 53.8 ± 3.6 mm, FS: 18.7 ± 2.8% vs. 18.3 ± 1.3% before and 24 weeks after an administration of teprenone) in comparison with the patients without the teprenone treatment (the plasma BNP levels: 248 ± 26 pg/ml vs. 238 ± 18 pg/ml, LVDd: 65.1 ± 1.3 mm vs. 66.7 ± 1.5 mm, LVDs: 53.6 ± 1.4 mm vs. 55.1 ± 1.2 mm, FS: 17.7 ± 1.3% vs. 17.2 ± 1.0% at observation time of 0 and 24 weeks). The frequency of readmission because of worsening of CHF was identical between the teprenone and control groups (2 [20%] and 2 [20%]).

DISCUSSION

In the present study, we demonstrate that the blockade of histamine H_2 receptors favors the improvements of the pathophysiology of CHF via retrospective and prospective clinical trials. These conclusions propose the novel findings that histamine that stimulates histamine H_2 receptors is one of the neurohumoral factors for the worsening of CHF, and that the blockade of histamine H_2 receptors becomes the novel strategy for the treatment of CHF.

Histamine in failing hearts. We have shown that histamine release is augmented in the ischemic myocardium compared with the non-ischemic myocardium in dogs (unpublished data). When the mast cells that store histamine are stimulated by ischemia or mechanical stress, mast cells actively release histamine. There are reports that mast cells are found in the human heart (19) and have been implicated in cardiovascular diseases (15,20,21). Indeed, the increase of mast cells have been observed in the hearts of patients with hypertrophy (22), dilated cardiomyopathy, ischemic cardiomyopathy (23), and ischemia/reperfusion (24), and the infarction-related coronary arteries (25). Furthermore, histamine is present in high concentrations in cardiac tissues in most animal species, including humans (10,26,27), and its release from cardiac stores and the subsequent actions on the heart may be of importance in the pathophysiology of heart disease. These lines of evidence agree with the present observation that the blockade of histamine H_2 receptors in failing hearts has an impact on the pathophysiology of CHF.

The role of histamine receptors in failing hearts. The histamine receptors (H_1, H_2, H_3, and H_4) are all G protein-coupled molecules, and they transduce extracellular signals via Gq, Gs, and Gi/o, respectively (5,6,28). Specifically, histamine H_2 receptors are linked to Gs proteins that facilitate the production of cyclic adenosine monophosphate (AMP) as beta-adrenoreceptors are (29). Histamine H_2-receptor-stimulated cAMP accumulation or adenylyl cyclase activator has been demonstrated in a variety of tissues including gastric cells (10,30), vascular smooth muscle cells (31), brain (10,32), and cardiac tissue (10,33). Beta-adrenoreceptor blockers are known to be cardioprotective in failing hearts because the accumulation of cyclic AMP after the activation of beta-adrenoreceptors enhances both myocardial contractility and oxygen consumption, which deteriorates heart function in patients with CHF (34,35). In addition, it has been reported that histamine is a powerful vasoconstrictor in atherosclerotic coronary arteries (36), which may locally provoke coronary spasm and thus contribute to the onset of myocardial infarction (23). The importance of beta-adrenoreceptor blockers depends on the presence of both catecholamine and beta-adrenoreceptors in the heart. Therefore, because histamine H_2 receptors and histamine are located in failing human hearts, it is likely that blockers of histamine H_2 receptors are as cardioprotective against failing hearts as beta-adrenoreceptor blockers are.

Because famotidine decreases both blood pressure and heart rate, this may improve the pathophysiology of CHF. Indeed, the reduction of afterload or preload and heart rate seems to be an important factor in the treatment of CHF. This is also the case in either beta-adrenoreceptor blockers or ACE inhibitors in patients with heart failure. Either beta-adrenoreceptor blockers or ACE inhibitors are still effective independent of the reduction of loading condition to the heart, because they inhibit the signal transduction for deterioration of cardiac function. Because histamine increases cyclic AMP levels in the cardiomyocytes via histamine H_2 receptors, famotidine may be beneficial through both load-reduction-dependent and -independent mechanisms.

Clinical importance. Beta-adrenoreceptor blockers have been shown to be effective for treating ischemic heart diseases and heart failure (37), and histamine receptor blockers are similar to beta-adrenoreceptor blockers. Histamine plays an important role in the regulation and malregulation of cardiac and coronary function. Furthermore, the histamine receptor blockers such as famotidine that are used for peptic ulcers or GERD all over the world could be used for ischemic heart diseases. Furthermore, beta-adrenoreceptor blockers ameliorate the severity of heart failure, and histamine receptor blockers may be beneficial for patients with CHF. However, we should note that the 3 H_2 receptor blockers administered for 7 days at clinical dosages had no significant effect on left ventricular systolic function, aerobic metabolic performance, or exercise capacity in men with class II or III stable CHF (38). This suggests that a relatively long-term administration of histamine receptor blockers is necessary to mediate the cardioprotective effects of histamine receptor blockers in patients with CHF as a relatively long-term administration of beta-adrenoreceptor blockers is necessary for the treatment of CHF (37).

Moreover, because famotidine was administered in addition to the aggressive treatments with beta-adrenoreceptors blockers, ACE inhibitors, and diuretics, and we proved that famotidine further improves the pathophysiology of CHF, it is possible to develop famotidine for the drug of CHF, although we need to plan and perform a large-scale clinical trial for the investigation of the effects of famotidine on CHF. We also need to clarify the best dose of famotidine for the treatment of CHF.

Study limitations. The present study has several limitations that we need to pay attention to. First of all, the first part of the data was obtained from the retrospective analysis, and seemed to be influenced by many factors, although Table 1 showed low BNP levels and low ventricular volumes in the famotidine group suggested the preventive effects of famotidine on cardiac remodeling. To strengthen the hypothesis obtained by the retrospective study, we performed the prospective analysis using either famotidine or teprenone.

The second limitation is that the second part of the study was an open-labeled, randomized trial using small sampling size. However, to decrease these weaknesses, we used the objective end points such as the plasma BNP levels and left ventricular dimensions, and we also tried to exclude the subjective scope of the assessment of NYHA functional classification.

Third, the severities of pathophysiology of CHF in the retrospective and prospective studies were different. The severity of CHF in enrolled patients in prospective study is higher than that in the retrospective study. This is because we enrolled the patients from the different protocols. Nevertheless, because both studies suggest that famotidine is effective for patients with CHF, we may be able to suggest the beneficial effects of famotidine to treat patients with CHF.

Fourth, if teprenone could be deleterious to the pathophysiology of CHF, famotidine seemed to be beneficial compared with teprenone even if famotidine has no beneficial effects on CHF. Before planning the present study, we tested the effects of pathophysiology of CHF, and we found that teprenone has no beneficial or deleterious effects on CHF in the present study.

Fifth, either famotidine or teprenone may directly affect the plasma half-life or excretion of BNP. If this is the case, the plasma BNP levels may be altered independent of the improvements of CHF. We cannot deny this possibility, however, because left ventricular dimension becomes smaller in the famotidine group, suggesting that famotidine is beneficial for the heart of CHF patients.

Sixth, we should notice that an interaction of gastritis with heart failure could confound their conclusion regarding the effect of histamine blockade. Indeed, it may be still possible to consider that famotidine improves cardiac function via an improvement of GERD if GERD worsens CHF, because we have no positive or negative data to link GERD and CHF. We should investigate this possibility to explain the effects of H_2 receptor blockers on CHF in further study.

In summary, despite these limitations, we proposed the hypothesis that H_2 receptor blockers are effective for the treatment of CHF, and we need to verify the beneficial effects of H_2 receptor blockers such as ranitidine or cimetidine as well as famotidine in CHF patients with a large-scale trial.

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Blockade of Angiotensin II Receptors Reduces the Expression of Receptors for Advanced Glycation End Products in Human Endothelial Cells

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Rapid Communication

Blockade of Angiotensin II Receptors Reduces the Expression of Receptors for Advanced Glycation End Products in Human Endothelial Cells

Masashi Fujita, Hiroko Okuda, Osamu Tsukamoto, Yoshihiro Asano, Yulin Liao Akio Hirata, Jiyoong Kim, Takeshi Miyatsuka, Seiji Takashima, Tetsuo Minamino, Hitonobu Tomoike, Masafumi Kitakaze

Objectives—Receptors for advanced glycation end products (RAGEs) play crucial roles in atherogenesis. Because tumor necrosis factor α (TNFα) is expressed and upregulates RAGE expression in atherosclerotic lesions, the TNFα-RAGE interaction might be involved in the inflammatory process of atherogenesis. On the other hand, an angiotensin II type-1 receptor blocker (ARB), widely used as an antihypertensive drug, has been reported to have also antiatherosclerotic effects. Thus we investigated whether an ARB exerts antiatherosclerotic effects via inhibiting the TNFα-RAGE interaction.

Methods and Results—Stimulation of human endothelial cells with candesartan as well as olmesartan decreased TNFα-induced RAGE expression in both mRNA and protein levels along with the decrease in the activity of nuclear factor κB and the expression of inflammatory mediators such as vascular cell adhesion molecule (VCAM)-1. Both candesartan and olmesartan inhibited the binding of nuclear factor κB to the RAGE gene promoter. Furthermore, gene silencing of RAGE by RNA interference decreased the expression of TNFα-induced VCAM-1 in both mRNA and protein levels.

Conclusions—RAGE contributes at least partially to the TNFα-induced VCAM-1 expression in both mRNA and protein levels. Blockade of angiotensin II receptors might exert antiatherosclerotic effects via reducing TNFα-RAGE interaction. ***(Arterioscler Thromb Vasc Biol.* 2006;26:e138-e142.)**

Key Words: angiotensin II type-1 receptor blocker (ARB) ■ receptors for advanced glycation end products (RAGEs) ■ endothelial cell

The cell surface receptor for advanced glycation end products (RAGEs) is a multiligand member of the immunoglobulin superfamily of molecules and has been reported to play crucial roles in atherogenesis.[1] Engagement of RAGEs in endothelial cells leads to the increase in the expression of inflammatory mediators such as monocyte chemoattractant protein (MCP)-1 or vascular cell adhesion molecule (VCAM)-1.[2-5] Because tumor necrosis factor (TNF)-α is expressed and upregulates RAGE expression in atherosclerotic lesions,[6,7] the TNFα-RAGE interaction might be involved in the mechanisms of the inflammatory process of atherogenesis.

On the other hand, an angiotensin II type-1 receptor blocker (ARB), widely used as an antihypertensive drug, has been reported to have also antiatherosclerotic such as attenuating neointimal formation, decreasing vascular smooth muscle cell (VSMC) proliferation and diminishing vascular inflammation.[8]

Considering these results, we hypothesized that an ARB has antiatherosclerotic effects via inhibiting TNFα-RAGE interaction, thus leading to the decrease in the inflammatory process of atherosclerosis. We tested this hypothesis using human endothelial cells.

Materials and Methods

Cell Culture

Human umbilical vein endothelial cells (HUVECs) were purchased from Biochrom and cultured at 37°C, under a humidified atmosphere

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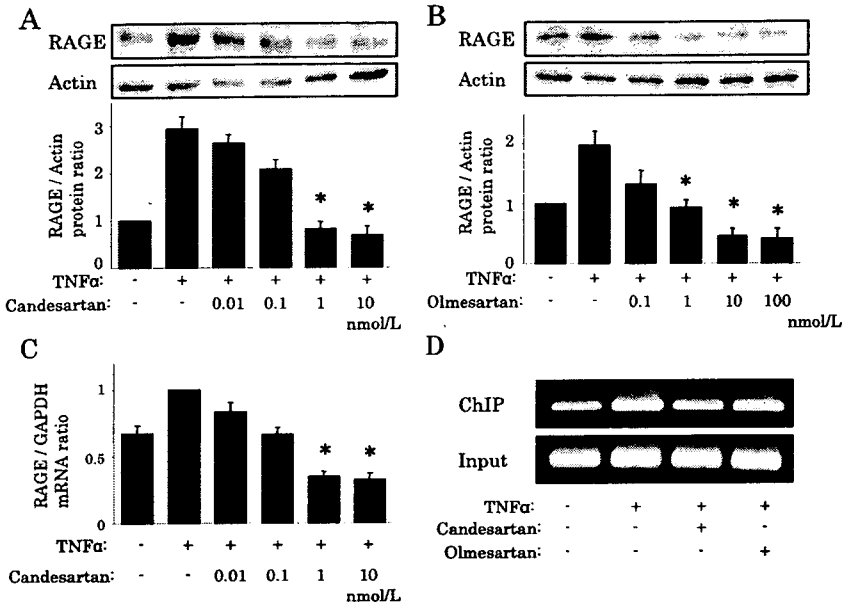

Figure 1. A and B, (upper panel) Representative Western blot analysis of RAGE proteins in HUVECs. (lower panel) Quantitative analysis of RAGE proteins by densitometry. Values are normalized to controls., C, Quantitative real-time PCR measurement of RAGE mRNA levels normalized GAPDH mRNA. D; ChIP assay. *$P<0.05$ vs TNFα

containing 5% CO_2 in EBM-2 medium (Clonetics) supplemented with EGM-2 Single Quotes containing 10% FBS, human fibroblast growth factor-1, vascular endothelial growth factor, ascorbic acid, heparin, and human epidermal growth factor and GA-1000. These cells were used at passages 2 to 10 for all experiments.

Western Blot Analysis

Standard Western blot analysis on total cell lysates was performed using mouse anti-RAGE, VCAM-1, IkBα antibodies (Santa Cruz). Candesartan and olmesartan were gifts from Takeda Pharm Co Ltd (Osaka, Japan) and Sankyo Co Ltd (Tokyo, Japan), respectively. To ensure equal loading of intact protein, membranes were stripped and restained with antibodies against actin. Densitometric analysis was performed using Scanning Imager (Molecular Dynamics).

Quantitative Real-Time Polymerase Chain Reaction

Total RNA from HUVECs was extracted using RNA-Bee-RNA Isolation Reagent (Tel-Test). Then 1000 ng of total RNA was reverse transcribed and amplified using an Omniscript RT Kit (Qiagen) according to the protocol of the manufacturer. Oligonucleotide primers and TaqMan probes for human RAGE (assay no. Hs00153957_m1), VCAM-1 (Hs00174239_m1), and GAPDH were designed and purchased from Applied Biosystems. Quantitative real-time PCR was performed with an ABI PRISM7700 Sequence Detection System (Applied Biosystems) by the relative standard curve method.

Chromatin Immunoprecipitation Assay

Either TNFα (10 ng/mL) or vehicle was exposed to HUVECs with and without either candesartan (1 nmol/L) or olmesartan (10 nmol/L) for 2 hours. ChIP assays were performed with a chromatin immunoprecipitation (ChIP) assay kit (Upstate Biotechnology) according to the protocol of the manufacturer with some modifications. The primers for the nuclear factor (NF)-κB–binding site in the RAGE gene promoter were: forward primer, 5′-GGAGGAGGTTGCAAAAAGCCAGAT-3′; reverse primer, 5′-CATCACACTTCCAACCTGTCCCCA-3′.

NF-κB p65 Transcription Factor Assay

The activation of NF-κB binding to the nucleus of HUVECs treated with and without TNFα (10 ng/mL) in the absence or presence of either candesartan (1 nmol/L) or olmesartan (10 nmol/L) was determined using the nonradioactive NF-κB p65 transcription factor assay kit (Chemicon International Inc) according to the instructions provided. Nuclear protein extracts were also prepared according to the protocol of the manufacture.

Gene Silencing via RNA Interference

HUVECs were seeded into P6 dishes coated with human fibronectin (Biocoat, BD-Falcon) and grown until ≈70% to 80% confluence, followed by transfection with 30 pmol of the negative control sequence or RAGE-specific small interference RNA (siRNA) duplex using Lipofectamine 2000 (Invitrogen) according to the instructions of the manufacturer. The following siRNA oligonucleotides for this study were purchased from Dharmacon: human RAGE [1] siRNA (sense, 5′-GCC AGA AGG UGG AGC AGU A-3′; antisense, 5′-UAC UGC UCC ACC UUC UGG C-3′); siRNA human RAGE [2] (sense, 5′-CCU CAA AUC CAC UGG AUG A-3′; antisense, 5′-UCA UCC AGU GGA UUU GAG G-3′). As a negative control, cells were transfected with siControl Non-Targeting siRNA No. 1 (Dharmacon). At 24 hours after transfection quantitative real-time polymerase chain reaction (PCR), 48 hours after transfection Western blot analysis were performed.

Statistical Analysis

Results of the experimental studies are reported as the means±SE. Differences were analyzed by ANOVA followed by the appropriate post hoc test. A probability value of <0.05 was regarded as significant.

Results

ARB Reduces the Expression of RAGE Protein and mRNA in Human Endothelial Cells

Because TNFα is known to increase endothelial RAGE protein expression, we first examined the effect of candesartan on TNFα-induced RAGE protein expression by Western blot analysis. Stimulation of HUVECs with TNFα (25 ng/mL) for 24 hours led to a 2.9±0.5-fold increase in cell surface RAGE protein expression compared with control ($P<0.05$, n=4), and, furthermore, concomitant treatment with candesartan significantly reduced the expression of TNFα-induced RAGE protein in a concentration-dependent manner with a maximal reduction at 1 nmol/L candesartan (Figure 1A). To confirm that this effect is not specific for candesartan alone, we checked the effects of other ARB,