Role of Endothelin in Deterioration of Heart Failure Due to Cardiomyopathy in Hamsters

Increase in Endothelin-1 Production in the Heart and Beneficial Effect of Endothelin-A Receptor Antagonist on Survival and Cardiac Function

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Background—We previously reported that chronic endothelin (ET) receptor blockade ameliorated the survival rate and cardiac hemodynamics in rats with chronic heart failure (CHF) due to myocardial infarction. However, it remains unclear whether ET-1 is involved in the pathophysiology of cardiomyopathy, which is one of the major causes of CHF. Accordingly, we investigated the production of ET-1 in the heart and the effect of chronic ETA receptor blockade on survival rate and cardiac function in the Bio 14.6 hamster, which is an idiopathic model of CHF caused by cardiomyopathy.

Methods and Results—We used 52-week-old Bio 14.6 cardiomyopathic hamsters and age-matched F1b normal hamsters. The expression of preproET-1 mRNA and the ET-1 level in the hearts were markedly higher in the cardiomyopathic hamsters than in the normal hamsters. The cardiomyopathic hamsters showed severe CHF, illustrated by lower left ventricular (LV) +dP/dt/P\text{max} and right ventricular (RV) +dP/dt/P\text{max} and by higher LV end-diastolic pressure (EDP), RVEDP, and central venous pressure compared with the normal hamsters. Long-term (9 weeks) treatment with an ETA antagonist (TA-0201, 1.3 mg \cdot kg\textsuperscript{-1} \cdot d\textsuperscript{-1}) markedly increased survival of cardiomyopathic hamsters (untreated, 16%; TA-0201–treated, 65.2%; P<0.001). After 6 weeks of treatment, LV +dP/dt/P\text{max} and RV +dP/dt/P\text{max} were significantly higher and LVEDP and RVEDP were lower in the TA-0201–treated group than in the untreated group, suggesting that chronic TA-0201 treatment effectively prevented deterioration of cardiac dysfunction.

Conclusions—In the cardiomyopathic hamsters with CHF, the production of ET-1 in the heart was markedly increased, and chronic ETA receptor blockade greatly ameliorated survival and cardiac dysfunction. These results suggest that ET-1 plays an important role in the deterioration of CHF caused by cardiomyopathy, and ET\text{A} antagonists may exert therapeutic effects in CHF due to cardiomyopathy. (Circulation. 1999;99:2171-2176.)

Key Words: endothelin ■cardiomyopathy ■heart failure

We and other groups have reported an increase in plasma levels of endothelin (ET)-1 in patients\textsuperscript{1-3} and experimental animals\textsuperscript{4,5} with chronic heart failure (CHF). It has been reported that plasma ET-1 level is a major predictor of mortality in patients with CHF.\textsuperscript{6} ET-1 is produced not only by endothelial cells\textsuperscript{7} but also by cardiac tissues, such as cardiac myocytes\textsuperscript{8} and fibroblasts.\textsuperscript{9} In cardiac myocytes, mechanical stretching\textsuperscript{10} and some neurohumoral factors (eg, angiotensin II and norepinephrine)\textsuperscript{11,12} stimulate the production of ET-1. Moreover, ET-1 has direct effects on contractile function,\textsuperscript{13} protein synthesis,\textsuperscript{14,15} and electrophysiological events\textsuperscript{16,17} in cardiac myocytes, and these effects are mediated primarily by ETA receptors. These observations suggest that increased ET-1 production and subsequent activation of ETA receptors contribute to the pathophysiological changes in CHF.

We previously reported that the plasma ET-1 level and production of ET-1 in the heart were markedly increased in CHF rats with myocardial infarction induced by coronary ligation.\textsuperscript{3} We also showed that long-term treatment with BQ-123, an ETA antagonist, greatly improved the survival rate of rats with CHF due to myocardial infarction.\textsuperscript{18} This beneficial effect of BQ-123 was accompanied by significant amelioration of left ventricular (LV) dysfunction and prevention of unfavorable ventricular remodeling.\textsuperscript{18}
However, the pathogenesis of congestive heart failure is diverse. Not only ischemic heart disease, for which a coronary-ligated rat serves as a model, but also cardiomyopathy constitutes a primary part of the causes of CHF. Therefore, investigations of the roles of ET-1 in a cardiomyopathic CHF model are valuable to understand the efficacy and mechanism of ET antagonists for the treatment of CHF due to cardiomyopathy. The Bio 14.6 hamster, a model of idiopathic cardiomyopathy, is one of the representative models of CHF due to cardiomyopathy. The pathogenesis of the disease state is not fully understood, but this model spontaneously develops cardiac necrosis, hypertrophy, and finally progression to dilatation and CHF in the later stages. Therefore, this model is appropriate for examining the effect of long-term treatment with drugs on the prognosis of CHF due to cardiomyopathy. However, either production of ET-1 in the heart or a role of ET-1 in the pathophysiology of this cardiomyopathic CHF model has not been investigated yet. We designed the present study to resolve these questions using an ETA receptor antagonist, TA-0201.

Methods

Animals and Study Protocols
We used male Bio 14.6 cardiomyopathic hamsters and F1b normal hamsters (Charles River, Yokohama, Japan). Hamsters were individually housed with a 12-hour light/dark cycle and received a powdered diet (CE-7, Nihon Clea) and tap water ad libitum. Hamsters were anesthetized with thiobutabarbital (Silenus, CE-7, Nihon Clea) and tamped water ad libitum. The animals were monitored for signs of "wet tail," a fatal diarrhea in rodents that occurs frequently in cardiomyopathic hamsters. Based on these criteria, 1 of 56 cardiomyopathic hamsters was excluded during the course of the study. Based on these criteria, 1 of 56 cardiomyopathic hamsters was excluded during the course of the study.

First Series of Experiments

Measurement of Cardiac Functions and Tissue Sampling
Measurements of cardiac functions were performed by a minor modification of the method described previously. Bio 14.6 and F1b hamsters (52 weeks old) were anesthetized with thiobutabarbital 100 mg/kg IP. A catheter (SP-10) was inserted into the right carotid artery to measure arterial blood pressure and was advanced into the LV for evaluating LV +dP/dt/P max. Subsequently, another catheter was inserted into the right jugular vein for the recording of central venous pressure (CVP) and was advanced into the right ventricle (RV) to measure RV +dP/dt/P max. After measurements of cardiac functions, blood samples were collected and stored at −80°C. The heart was excised and divided into the RV and the LV including the septum. After each ventricle was weighed, all samples were frozen in liquid nitrogen and stored at −80°C.

Sandwich Enzyme Immunoassay for Determination of Heart and Plasma ET-1 Levels
The LV ET-1 level was determined as previously described in our publications. Sandwich enzyme immunoassay (EIA) for ET-1 was carried out with immobilized mouse monoclonal antibody AwETN40, which recognizes the N-terminal portion of ET-1, and peroxidase-labeled rabbit anti–ET-1 C-terminal peptide 15-21 Fab′. The assay for ET-1 did not cross-react with ET-3 or big ET-1 (cross-reactivity <0.1%). Plasma ET-1 concentration was also measured by a sandwich EIA.

Reverse Transcription and Polymerase Chain Reaction to Evaluate the Level of PreproET-1 mRNA in the LV
Extraction of total RNA from the LV and analysis of preproET-1 mRNA expression by reverse transcription and polymerase chain reaction (RT-PCR) were performed as described previously. Total RNA was reverse-transcribed by avian myeloblastosis virus reverse transcriptase. The gene-specific primer pair of preproET-1 was designed on the basis of the partial sequence of hamster preproET-1. The expression of GAPDH mRNA was also determined as an internal control. The gene-specific primer pair of GAPDH was designed on the basis of the cDNA sequence for hamster GAPDH. The sequences of the oligonucleotides were as follows: preproET-1 (sense): 5'-CGAGCTGAGAATGAAGGGAGAG-3'; preproET-1 (antisense): 5'-GCCCTTCTGATGGGTTTGGCC-3'; GAPDH (sense): 5'-CTCAACTACATGGTGACTC-3'; and GAPDH (antisense): 5'-CTTCCACATGCAAAAGT-3'.

The reaction cycles of PCR were performed in a range that shows a linear correlation between the amount of cDNA and the yield of PCR products. The amplified PCR product was electrophoresed on a 1.2% agarose gel, stained with ethidium bromide, visualized by UV transilluminator, and photographed. The photograph was scanned by a scanner (CanoScan 600; Canon) and quantified by computer with MacBas software (Fuji Film).

Second Series of Experiments

Effect of TA-0201, an ETA Antagonist, on Survival Rate of Cardiomyopathic Hamsters
Bio 14.6 hamsters (52 weeks old) were used in the study of survival rate. Hamsters were randomized to receive either placebo (n = 25) or TA-0201 (n = 24). TA-0201 was mixed into their diet at a concentration of 30 ppm. The daily dose of TA-0201 in the treated group was 0.1 mg·kg−1·d−1 and the plasma concentration of TA-0201 was 63.9 ± 30.4 ng/mL (n = 7). We reported that TA-0201 is an orally active ET A antagonist. In the binding study using human cloned ET receptors, TA-0201 showed higher affinity for ET A receptors (Kd = 0.015 mmol/L) than for ET B receptors (Kd = 41 mmol/L).

Throughout the experiment, each hamster was inspected for signs of "wet tail," a fatal diarrhea in rodents that occurs frequently in hamsters. Hamsters that died of wet tail were excluded from the study. Based on these criteria, 1 of 56 cardiomyopathic hamsters was excluded during the course of the study.

Effect of TA-0201 on Cardiac Functions of Hamsters
We also evaluated the effect of chronic treatment with TA-0201 on cardiac functions of hamsters. Hamsters were randomized to receive either placebo or TA-0201. Noncardiac death was observed in 1 animal during the course of the study. After 6 weeks of treatment, all of the surviving hamsters were anesthetized with thiobutabarbital 100 mg/kg IP, and cardiac functions were measured as described above.

Statistical Analysis
All data except the survival rate are expressed as mean ± SEM. Comparison of survival in untreated and TA-0201–treated cardiomyopathic hamsters was analyzed by a log-rank test with the Kaplan-Meier method. Plasma ET-1 levels in cardiomyopathic and normal hamsters with or without TA-0201 were examined by use of 2-way ANOVA with contrasts. The analytical method used in other studies was the unpaired t test. Differences were considered significant at the level of P < 0.05.

Results

First Series of Experiments
Cardiac Function and Tissue Weights
Table 1 shows mean blood pressure (MBP), heart rate (HR), and cardiac functions measured in anesthetized hamsters. In the 52-week-old cardiomyopathic hamsters, MBP, HR, LV +dP/dt/P max, and RV +dP/dt/P max were significantly lower.
than in age-matched normal hamsters. In addition, LV end-diastolic pressure (LVEDP), RVEDP, and CVP were markedly elevated in the cardiomyopathic hamsters. These data indicated that the cardiomyopathic hamsters in 52 weeks had developed severe cardiac dysfunction and CHF. LV and RV mass indices for body weight (LV/BW and RV/BW) were much higher in the cardiomyopathic hamsters than in the normal hamsters (Table 1).

**Table 1. Hemodynamic Measurements and Tissue Weights in 52-Week-Old F1b and Bio 14.6 Hamsters**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>F1b (n=10), Normal</th>
<th>Bio 14.6 (n=10), Cardiomyopathic</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP, mm Hg</td>
<td>132±5</td>
<td>71±6†</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>342±9</td>
<td>306±14*</td>
</tr>
<tr>
<td>LV +dP/dt/P max, s⁻¹</td>
<td>267±6</td>
<td>107±7†</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>2.3±0.6</td>
<td>13.4±2.1†</td>
</tr>
<tr>
<td>RV +dP/dt/P max, s⁻¹</td>
<td>250±12</td>
<td>148±25†</td>
</tr>
<tr>
<td>RVEDP, mm Hg</td>
<td>0.0±0.0</td>
<td>1.8±0.8*</td>
</tr>
<tr>
<td>CVP, mm Hg</td>
<td>0.1±0.1</td>
<td>2.5±0.8†</td>
</tr>
<tr>
<td>BW, g</td>
<td>151±6</td>
<td>117±7†</td>
</tr>
<tr>
<td>LV/BW, mg/g</td>
<td>2.44±0.03</td>
<td>3.64±0.17†</td>
</tr>
<tr>
<td>RV/BW, mg/g</td>
<td>0.67±0.02</td>
<td>1.02±0.04†</td>
</tr>
<tr>
<td>Lung/BW, mg/g</td>
<td>4.27±0.11</td>
<td>4.66±0.20</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*P<0.05, †P<0.01 vs F1b.

PreproET-1 mRNA Expression and Tissue ET-1 Level in the LV and Plasma ET-1 Level

The expression of preproET-1 mRNA was markedly higher in cardiomyopathic hamsters than in normal hamsters (Figure 1). The values of band density of PCR products for GAPDH mRNA did not differ between the 2 groups. As shown in Figure 2A, the peptide ET-1 level in the LV of cardiomyopathic hamsters was ~3.2-fold higher than that of normal hamsters. The plasma ET-1 concentration of the cardiomyopathic hamsters was also ~1.7-fold higher than that of normal hamsters (Figure 2B). Plasma ET-1 level was significantly correlated with LV +dP/dt/P max (r=−0.81, P<0.001) in the hamsters. The peptide level of ET-1 in the LV was also significantly correlated with LV +dP/dt/P max (r=−0.67, P<0.01).

Second Series of Experiments

Effect of Chronic Administration of TA-0201 on Survival Rate of Cardiomyopathic Hamsters

Chronic treatment with TA-0201 markedly increased the survival rate of the cardiomyopathic hamsters (Figure 3). At
At the end of the experiment (after 9 weeks of treatment), the survival rates of cardiomyopathic hamsters were 16% in the untreated group and 65.2% in the TA-0201–treated group (Figure 3). The survival rate of the treated group was significantly higher than that of the untreated group ($P < 0.001$).

### Effect of Chronic Administration of TA-0201 on Cardiac Function and Plasma ET-1 Levels in Cardiomyopathic Hamsters

Cardiac function was measured in anesthetized hamsters after 6 weeks of treatment with or without TA-0201. At this point, the survival rate of the TA-0201–treated cardiomyopathic hamsters was significantly higher than that of the untreated hamsters (82.6% versus 50%, $P < 0.05$).

Chronic administration of TA-0201 did not affect MBP or HR in cardiomyopathic hamsters (Figure 4). On the contrary, TA-0201–treated hamsters showed significantly higher LV $+dP/dt/P_{\text{max}}$ (Figure 4) and RV $+dP/dt/P_{\text{max}}$ (Figure 5) compared with the untreated hamsters. In addition, LVEDP (Figure 4), RVEDP (Figure 5), and CVP (Figure 5) were significantly lower in the TA-0201–treated compared with the untreated hamsters. With regard to the tissue weights, RV/BW and lung/BW were also significantly lower in the TA-0201–treated than in the untreated hamsters (Table 2).

In contrast to cardiomyopathic hamsters, TA-0201 showed no significant effects on hemodynamics and tissue weights in F1b hamsters (data not shown).

### Discussion

The present study clearly demonstrated that the production of ET-1 is markedly increased in the LV of Bio 14.6 cardiomyopathic hamsters at a stage of CHF. The chronic administration of an ETA antagonist, TA-0201, markedly improved the survival rate of cardiomyopathic hamsters. In addition, after 6 weeks of treatment, TA-0201 effectively prevented (1) the increase in LVEDP, RVEDP, and CVP; (2) the decrease in LV $+dP/dt/P_{\text{max}}$ and RV $+dP/dt/P_{\text{max}}$; and (3) RV hypertrophy. These results suggest that chronic ET blockade improves the survival rate and ameliorates both LV and RV dysfunction in cardiomyopathic hamsters. Therefore, it is strongly suggested that endogenous ET-1 plays an important role in the deterioration of CHF due to cardiomyopathy in hamsters.

Cardiomyopathy, as well as myocardial infarction, is one of the major causes of CHF. It has been reported that plasma ET-1 level is increased in patients with cardiomyopathy. To
date, however, the contribution of ET-1 in CHF caused by cardiomyopathy has not been investigated. Therefore, in the present study, we used Bio 14.6 cardiomyopathic hamsters (52 weeks old) to evaluate the role of ET-1 in CHF due to cardiomyopathy. At this age, not only plasma ET-1 concentration but also ET-1 production in the LV was significantly higher in the cardiomyopathic hamsters than in the age-matched normal hamsters, suggesting that ET pathway is activated in the cardiomyopathic hamsters. We also observed that LV $+dP/dt/P_{max}$ was markedly lower in the cardiomyopathic than in the normal hamsters. The LV $+dP/dt/P_{max}$ was negatively correlated with both plasma and LV ET-1 level in the hamsters. Plasma ET-1 concentration is well correlated with the clinical class of heart failure, New York Heart Association functional class in patients with chronic CHF.3

Taken together, the increased plasma ET-1 concentration in the cardiomyopathic hamsters may reflect the severity of cardiac dysfunction.

The long-term administration of an ET$_A$ antagonist, TA-0201, greatly improved survival of the cardiomyopathic hamsters. Indeed, the survival rate was 16% in the untreated cardiomyopathic hamsters and 65.2% in the TA-0201–treated hamsters. To the best of our knowledge, this is the first study to describe the beneficial effect of ET blockade on survival of cardiomyopathic hamsters at the CHF stage. We have already reported the beneficial effect of BQ-123, another ET$_A$ antagonist, in rats with CHF induced by coronary ligation.18 The administration of BQ-123 almost doubled the number of surviving rats with CHF; the survival rate of CHF rats treated with BQ-123 was 85%, compared with 43% for the untreated CHF rats.18 Therefore, ET antagonists may be beneficial for the treatment of CHF caused not only by ischemic heart disease but also by cardiomyopathy. We observed that LV $+dP/dt/P_{max}$ and RV $+dP/dt/P_{max}$ were markedly lower in the 52-week-old cardiomyopathic hamsters than in the age-matched normal hamsters. Moreover, LVEDP, RVEDP, and CVP were markedly elevated in the cardiomyopathic hamsters compared with the normal hamsters. These results indicate that the cardiomyopathic hamsters at 52 weeks old already have severe cardiac dysfunction and are in an advanced stage of CHF. Therefore, the beneficial effect of TA-0201 on the survival rate of cardiomyopathic hamsters suggests that ET$_A$ antagonists have a therapeutic potential for CHF even when treatment is initiated at an advanced stage of CHF.

With regard to cardiac function, LV and RV performances in the untreated 58-week-old hamsters had progressively deteriorated compared with those in the 52-week-olds. The present study demonstrated that long-term treatment with TA-0201 significantly prevented the progression of cardiac dysfunction. Thus, these results suggest that ET-1 contributes to the progression of CHF due to cardiomyopathy and that these effects of an ET$_A$ antagonist on cardiac hemodynamics might favorably contribute to the prolongation of life expectancy in cardiomyopathic hamsters.

In this study, long-term treatment with TA-0201, an ET$_A$ antagonist, did not affect the arterial pressure in the cardiomyopathic hamsters. This result is in accordance with our previous data that the administration of BQ-123 did not alter the arterial pressure in rats with CHF due to myocardial infarction.18 However, Mulder et al26 and Fraccarollo et al27 reported that long-term treatment with bosentan, an ET$_A$/ET$_B$ nonselective antagonist, lowers arterial pressure in rats with CHF due to myocardial infarction. One possible explanation for the discrepancy in effects on arterial pressure among ET antagonists is that the blockade of both ET$_A$ and ET$_B$ receptors by bosentan contributes to hypotension, because both ET$_A$- and ET$_B$-mediated mechanisms for vascular contraction exist in various vessels. Indeed, in spontaneously hypertensive rats, bosentan lowers arterial pressure,28 whereas an ET$_A$ antagonist shows no significant effect.29

Furthermore, TA-0201 showed no significant effect on plasma ET-1 levels after 6 weeks of treatment in hamsters. Other investigators have demonstrated that ET$_A$/ET$_B$ nonselective antagonists, such as bosentan, increased plasma ET-1 and have suggested that this increase was partly attributed to the blockade of ET$_B$ receptor, which is considered to be involved in ET-1 clearance.30,31 Therefore, it is likely that long-term treatment with an ET$_A$ antagonist does not affect ET-1 clearance in hamsters.

We clearly indicated the beneficial effects of an ET$_A$ antagonist on survival rate and cardiac function, and the mechanisms for this improvement should be elucidated. First, because ET-1 has direct toxic effects on cardiac myocytes,32 an increase in ET-1 production in the heart may lead to myocardial injury and may contribute to the progression of CHF. Second, because ET-1 exerts a hypertrophic effect on cardiac myocytes,14,15 it is likely that the upregulated ET-1 system in the heart contributes to the excessive hypertrophy of myocardium in cardiomyopathic hamsters. Third, ET-1 is known to induce ventricular arrhythmia,33 and high arrhythmogenicity of the ventricles was reported in cardiomyopathic hamsters.34 Therefore, it is likely that the inhibition of ET-1–induced arrhythmia by ET antagonists is beneficial to increasing the survival rate. Fourth, because it was reported that the acute administration of some ET antagonists caused a moderate peripheral vasodilation in humans,35 we cannot exclude the possibility that the inhibition of ET-1–induced vasoconstriction partly contributes to the beneficial effects of the ET$_A$ antagonist.

In conclusion, our study demonstrates for the first time that (1) the production of ET-1 in the LV was greatly increased in

![Figure 6. Plasma ET-1 levels after 6 weeks of treatment with TA-0201 in F1b hamsters (untreated, n=13; TA-0201–treated, n=13) and in Bio 14.6 hamsters (untreated, n=12; TA-0201–treated, n=19). Each column and bar represents mean±SEM.](image-url)
the cardiomyopathic CHF hamsters, and (2) the chronic treatment with an ET\textsubscript{A} antagonist markedly improved the survival, cardiac function, and hypertrophy of cardiomyopathic hamsters with advanced CHF. These observations suggest that ET-1 contributes to the progression of CHF due to cardiomyopathy and that an ET\textsubscript{A} antagonist has therapeutic potential for CHF due to cardiomyopathy.

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