Long-term treatment with neutral endopeptidase inhibitor improves cardiac function and reduces natriuretic peptides in rats with chronic heart failure

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Abstract

Objective: Increased secretion of atrial and brain natriuretic peptide (ANP and BNP) from hearts is known to exhibit favorable effects in patients and animals with heart failure, and inhibition of neutral endopeptidase (NEP), an enzyme that degrades ANP and BNP, may further increase these peptide levels. However, it is still unknown whether such elevation of the ANP and BNP may offer a therapeutic benefit to the progression of chronic heart failure (CHF). We examined the effects of ONO-9902, a novel NEP inhibitor, on changes in hemodynamic parameters, NEP activity and neurohumoral factors in rats with CHF induced by left coronary artery ligation (CAL).

Methods: Male Wistar rats (220–240 g) were subjected to induction of acute myocardial infarction by CAL. Rats were orally treated with ONO-9902 (300 mg / kg / day) from the 1st to 6th week after the operation. Hemodynamic and / or biochemical assessments were performed at the 1st and 6th weeks after the operation. Results: A single administration of ONO-9902 inhibited the plasma and kidney NEP activities and thereby further augmented the elevation of plasma ANP concentration in rats with CAL at the 1st week after the operation. In rats with CAL at the 6th week after the operation, the left ventricular end-diastolic pressure (LVEDP) increased and cardiac output index (COI) decreased as compared with those of sham-operated rats. These changes were accompanied by marked increases in the plasma ANP, BNP and endothelin-1 (ET-1). Chronic treatment with ONO-9902 attenuated the increase in LVEDP and the decrease in COI. These changes were associated with a decrease in plasma ANP, BNP and ET-1 concentrations. Conclusions: The results suggest that chronic treatment with NEP inhibitor improves depressed cardiac function in rats with CHF. ONO-9902 may offer a new and possible therapeutic approach in patients with CHF. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Heart failure; Hemodynamics; Natriuretic peptide; Nitric oxide; Endothelins; Remodeling

1. Introduction

It is well established that circulating levels of atrial and brain natriuretic peptide (ANP and BNP) are elevated in patients with chronic heart failure (CHF) in proportion to the severity of the disease [1,2]. Unlike vasoconstrictor peptides such as angiotensin II and endothelin-1 (ET-1), ANP and BNP have been shown to exhibit beneficial effects on systemic circulation in patients with CHF including dilatation of resistance vessels, natriuresis and diuresis [3]. It has been reported that infusion of ANP or BNP decreased both preload and afterload and increased cardiac output in patients with congestive heart failure whose plasma concentrations of ANP and BNP had already been high as compared with those of normal subjects [4–6]. These findings imply that only a high level of endogenous ANP and BNP would not improve the pathophysiology of heart failure and that an additional increase in these peptides may be necessary to elicit the improvement. However, the therapeutic potential of ANP and BNP themselves is limited because these peptides are inactive in an oral administration and are short-acting after intravenous administration. Thus, an orally active analogue of drugs that can inhibit their degradation would be expected to be useful for the therapy of CHF.

Neutral endopeptidase (NEP; also called enkephalinase,
CALLA/CD10, EC3.4.24.11) is a zinc-containing membrane-bound enzyme that is widely distributed in organs, particularly in the kidney [7] and lungs [8] with a high activity. NEP degrades ANP by cleaving the Cys7–Phe8 bond of the peptide [9]. Since NEP plays a major role in the clearance of ANP under pathological conditions where there is a higher plasma concentration as in CHF [10], NEP inhibition is regarded as a process of maintaining the biological activity of endogenous ANP by preventing its degradation. Therefore, inhibition of the ANP degradation by treatment with NEP inhibitor would be expected to exert beneficial effects on CHF because of amplification of the ANP action. Indeed, acute treatment with NEP inhibitors has been shown to produce therapeutically favorable responses, including reduction in systemic blood pressure [11], increases in cardiac output [12] and renal natriuretic action [13] and a decrease in the right atrial and pulmonary capillary wedge pressure [14] in various experimental models of heart failure or in patients with CHF. In contrast to the beneficial acute effects, treatment with candoxatril, a NEP inhibitor that is orally active prodrug of candoxatrilat, for 10 days failed to produce favorable hemodynamic effects in patients with CHF [15]. In addition, treatment with candoxatril for 4 weeks in the animal models of CHF failed to oppose cardiac hypertrophy [16]. There are, as far as we know, no reports concerning the benefit of long-term treatment with a NEP inhibitor for CHF. The present study was undertaken to examine whether long-term treatment with NEP inhibitor may produce beneficial effects in rats with CHF following left coronary artery ligation (CAL).

It is anticipated that chronic treatment with a NEP inhibitor, like acute treatment with a NEP inhibitor as described above [14], may increase plasma levels of ANP and BNP due to an inhibition of their degradation enzyme. Conversely, if the sustained elevation of circulating levels of these peptides is beneficial to CHF, long-term treatment with the NEP inhibitor may reduce the plasma ANP and BNP levels rather than elevate their levels as a result of the improvement of the pathophysiology of CHF. Thus, we also examined both acute and chronic effects of NEP inhibitor treatment on the plasma ANP and BNP concentrations in rats with CHF.

In this study, we used ONO-9902, as a novel NEP inhibitor, which was recognized as an anti-nociceptive agent due to its ability to inhibit enkephalinase [17]. However, its effect on cardiovascular function in CHF has not been clarified. This drug is an oral prodrug, which is hydrolyzed to release the active form, ONO-BB-039-02. The chemical structures of these compounds are shown in Fig. 1.

2. Methods

2.1. Animals

Male Wistar rats weighing 220–240 g (SLC, Hamamatsu, Japan) were used in the present study. The animals were fed with standard rat chow and tap water ad libitum, maintained at 23±1°C with a constant humidity of 55±5%, and acclimated with a cycle of 12 h of light and 12 h of darkness. All experiments were performed according to the Guidelines of Experimental Animal Care issued by Prime Minister’s Office of Japan, which is essentially in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No.85-23, revised 1996). The protocol of the study was approved by the Animal Care Committee of the Tokyo University of Pharmacy and Life Science.

2.2. Heart failure following left coronary artery ligation

Myocardial infarction was produced by CAL according to the method described previously [18]. Briefly, the animals were anaesthetized with pentobarbital sodium (45 mg/kg, i.p.), intubated and artificially ventilated with air. The $P_{\text{O}_2}$, $P_{\text{CO}_2}$ and pH values of the animal under surgery, when determined in a preliminary study, were 97.4±5.7 mmHg, 40.0±1.1 mmHg and 7.44±0.02 ($n = 6$), respectively. The skin was incised along the left sternal border, and the fourth rib was cut proximal to the sternum. The pericardial sac was perforated, and the heart was exteriorized through the intercostal space. The left coronary artery was ligated approximately 2 mm from its origin with a
monitored. The animals that showed a large Q wave (>1 mV) were considered to have developed acute myocardial infarction and were used for the following experiments. Among 124 animals that had undergone the operation, 35 died within 24 h and 9 within 1 week of the operation. The 80 remaining rats were used for the following studies. Sixty-four sham-operated (Sham) rats without CAL were treated similarly. Using these animals, we assessed acute and chronic effects of the NEP inhibitor. Experimental protocols and numbers of animals used for various series of experiments are shown in Fig. 2 and Table 1, respectively.

### 2.3. Treatment with ONO-9902

To assess the effects of acute treatment with ONO-9902, we orally treated the rats with a single dose of 300 mg/kg body weight of ONO-9902 7 days after CAL or sham operation. For evaluation of the effects of long-term treatment with ONO-9902, both rats with CAL and Sham rats were orally treated with 300 mg/kg body weight of ONO-9902 once daily for 5 weeks from the 1st to the 6th week after the operation. The drug solution in a volume of 1 ml/200 g body weight was administered into the stomach through a probe. The dose used in the present study was based on the in vivo anti-nociceptive effect of this drug on both somatic and visceral pain produced under visceral stress conditions [17].

### 2.4. Measurement of plasma and tissue NEP activities

To assess the effects of ONO-9902, we determined NEP activities in plasma, kidney and lungs at 24 h after the oral administration of ONO-9902 to rats with CAL and Sham rats. Under anesthesia with diethylether, blood was collected via the abdominal aorta, and the kidney and lungs were dissected. For determination of NEP activity, we employed HPLC-linked fluorimetric method using succinyl-Ala-Ala-Phe-aminomethylcoumarine (AMC) (Sigma, St. Louis, MO, USA) as a synthetic substrate for

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Table 1

<table>
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* Fifty rats at the 1st week after the operation were used for the acute study and 84 rats at the 6th week after the operation were used for the chronic study.
NEP and thiorphan (Sigma) as a NEP inhibitor. The principle of this method is similar to that described elsewhere [12,19]. To eliminate non-specific cleavage of the substrate, thiorphan at a concentration of 1 μM was added to the control tube. The AMC released was separated by reversed-phase HPLC from its substrate and intermediate products, and the fluorometric intensity was determined. NEP activity was calculated from the difference in AMC levels between the sample and control.

2.5. Measurement of plasma and tissue ANP, BNP and ET-1

To evaluate the acute effect of ONO-9902 on plasma concentrations of ANP and BNP, we used the rats with CAL and Sham rats at 1 week after the operation. For assessment of the chronic effect of ONO-9902 on plasma concentrations of ANP, BNP and ET-1, the rats with CAL and Sham rats, both with and without ONO-9902 treatment for 5 weeks, were used. At 24 h after the administration of ONO-9902, the plasma was sampled under anesthesia with diethylether from the abdominal aorta into a chilled tube containing aprotinin (300 kallikrein-inhibitable U/ml) and potassium EDTA (1.5 mg/ml blood). Plasma was separated by centrifugation at 2000 g for 10 min at 4°C and stored at −80°C until assayed. The heart was subsequently removed and rinsed in ice-cold normal saline. The scar area was removed from the left ventricle. The remaining left ventricle including the septum was sectioned from the heart, rapidly frozen in liquid nitrogen and stored at −80°C. ANP, BNP and ET-1 were extracted from the plasma by use of Sep-Pak C18 cartridges (Waters of Millipore, Milford, MA, USA) that had been preconditioned with methanol followed by 0.2 M sodium phosphate–citric acid, pH 7.0. Plasma samples were passed through the equilibrated cartridge. After washing with water, the cartridges were eluted with methanol–water (90:10, v/v). To determine the tissue ANP content, we ground the frozen non-infarcted part of the left ventricle to a fine powder with a mortar and pestle under liquid nitrogen cooling. The powder was homogenized in 1 M acetic acid containing 10 μl/ml pepstatin and immediately boiled for 10 min. After boiling and then chilling, the homogenate was centrifuged at 20000 g for 30 min at 4°C, and the supernatant fluid was stored at −80°C until used. ANP, BNP and ET-1 concentrations in the supernatant fluid were determined by the sandwich-enzyme immunoassay method (Peninsula Lab., San Carlos, CA, USA).

2.6. Measurements of hemodynamic parameters

In the first set of experiments, heart rate (HR), mean arterial pressure (MAP), left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), \(+dP/dt\) max and \(-dP/dt\) max of Sham and CAL animals with or without ONO-9902 treatment were measured according to the method described previously [18]. Six weeks after the operation, the animals were anesthetized with nitrous oxide–oxygen (3:1) and 2.5% halothane. Anesthesia was continued with a gas mixture of nitrous oxide–oxygen (3:1) containing 0.5% halothane at the flow-rate of 1.2 l/min through a mask loosely placed on the nose. The animals were warmed by an electronic panel heater to maintain their rectal temperature at 36–37°C. In a preliminary study, we analyzed the blood gas of the animal following the operation under the experimental conditions. The \(P_{O2}\), \(P_{CO2}\) and pH were 101.5±4.9 mmHg, 40.1±1.4 mmHg and 7.45±0.02 (n=5), respectively. A microtip pressure transducer (model SPG 320, Miller Instrument, Houston, TX, USA) was introduced into the ventricle through the right carotid artery to measure the respective LVSP and its \(dP/dt\) max by a pressure transducer (model AB-621-G, Nihonkohden, Tokyo, Japan) and a differentiator (model RD-601G, Nihonkohden). The MAP was measured by means of another pressure transducer (model DX-360, Nihonkohden) connected to a cannula placed into the right femoral artery. Measurement of HR was triggered from changes in MAP (model AT-601G, Nihonkohden). After equilibration for 10 min, the parameters were recorded on a thermal pen recorder (model RTA-1200, Nihonkohden).

In another set of experiments, aortic blood flow (AF) was measured and the cardiac output index (COI) was determined by the method described previously [18]. Six weeks after the operation, the Sham and CAL animals with or without ONO-9902 treatment were anesthetized with nitrous oxide–oxygen (3:1) and 2.5% halothane. Then each rat was intubated and artificially respirated with a gas mixture of nitrous oxide–oxygen (3:1) containing 0.5% halothane at the flow-rate of 3.2±0.3 ml/cycle (60 cycles/min). After thoracotomy, an electromagnetic flow meter with a diameter of 2–2.5 mm (model MFV-3100, Nihonkohden) was placed around the ascending thoracic aorta and the AF was then measured. During the measurement of AF, MAP was monitored through a cannula inserted into the femoral artery, and HR was measured via the arterial pressure recording in a manner similar to that described above. COI was calculated by dividing AF by body weight. In a preliminary study, we performed blood gas analysis on the experimental animals and found that the \(P_{O2}\), \(P_{CO2}\) and pH were 98.5±3.3 mmHg, 37.5±1.6 mmHg and 7.38±0.03 (n=5), respectively.

2.7. Measurements of myocardial infarct size

After measurements of hemodynamic parameters, 50 mM KCl solution was intravenously injected. The heart was isolated and sectioned into seven slices (1 mm thick) from the base to the apex of the heart in a plane parallel to the atrioventricular groove. The slices were stained at 37°C for 5 min with 1% 2,3,5-triphenyltetrazolium chloride in
physiological saline. The infarct areas were determined according to the planimetric method. The estimation was based on determination of the epimyal and endo-circumference of the infarcted myocardium as reported previously [20]. Six weeks after the operation, ten rats that had an infarct size of <35% of the left ventricle (six drug-treated and four untreated animals) were eliminated from the study.

2.8. Substances

ONO-9902, (4S)-4-[(2S)-benzyl-3-[(1RS)-1,3-dihydro-3-isobenzofuranyl-1-thio] propionylamino]-4-(N-phenylcarbamoyl)-butyric acid (Fig. 1) was kindly provided by Ono Pharmaceutical Co. Ltd. (Osaka, Japan).

2.9. Statistical analysis

Data are expressed as means±S.E.M. Statistical significance was estimated by two-way factorial ANOVA followed by Fisher’s PLSD method. Differences with a probability of <5% were considered significant (P<0.05).

3. Results

3.1. Effects of acute treatment with ONO-9902 on NEP activity and on plasma concentrations of ANP and BNP

To examine the degree of inhibitory potency of ONO-9902 on the NEP activity, we measured plasma and tissue NEP activities of rats with CAL and Sham rats following a single administration with ONO-9902 (Table 2). The plasma and renal NEP activities of rats with CAL were significantly reduced, and the pulmonary NEP activity tended to be decreased, on treatment with ONO-9902. In Sham rats, ONO-9902 significantly decreased plasma, renal and pulmonary NEP activities. These results indicate that ONO-9902 inhibited the NEP activity under ex vivo conditions irrespective of the presence or absence of CAL. Effects of acute treatment with ONO-9902 on changes in plasma ANP and BNP concentrations are shown in Fig. 3a and b, respectively. The plasma ANP level significantly increased in rats with CAL at the 1st week after the operation (106.2±14.7 vs. 34.0±5.4 pg/ml for Sham rats). This increase in the plasma ANP level was further enhanced by treatment with ONO-9902. Treatment with ONO-9902 tended to increase the plasma BNP in rats with CAL.

3.2. Effects of long-term treatment with ONO-9902 on changes in hemodynamic parameters

Effects of long-term treatment with ONO-9902 on changes in body and organ weights in rats with CAL and in Sham rats are shown in Table 3. Two-way ANOVA showed that the body weight was decreased whereas the lung weight, lung weight/body weight, right ventricular (RV) weight and RV weight/body weight were increased by CAL. The findings suggest that the rats with CAL exhibited pulmonary edema and right ventricular hypertrophy. Chronic treatment with ONO-9902 significantly attenuated the increases in lung weight and lung weight/body weight, whereas it did not affect the increase in heart weight of the rats with CAL. Myocardial infarct size in rats with CAL was not affected by the drug treatment (44±2 vs. 45±3% for untreated CAL group).

To determine whether the chronic inhibition of NEP may improve hemodynamic parameters, we examined the effects of long-term treatment with ONO-9902 on changes in HR, MAP, LVSP, LVEDP, +dP/dt max and −dP/dt max in rats with CAL and Sham rats (Table 4). Two-way ANOVA revealed that an increase in LVEDP of the CAL rats was significantly attenuated by long-term treatment with ONO-9902. Neither CAL nor treatment with ONO-9902 affected HR or MAP. The decreases in +dP/dt max and −dP/dt max tended to be restored by treatment with ONO-9902.

To determine whether the chronic inhibition of NEP may improve cardiac function, we assessed the effects of long-term treatment with ONO-9902 on changes in AF, COI, stroke volume index (SVI) and systemic vascular resistance (SVR) in rats with CAL and Sham rats in

<table>
<thead>
<tr>
<th>Neutral endopeptidase (NEP) activities</th>
<th>Sham (Untreated)</th>
<th>Sham (Treated)</th>
<th>CAL (Untreated)</th>
<th>CAL (Treated)</th>
<th>P (ANOVA)</th>
<th>P (untreated vs. treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma (nmol/ml/min)</td>
<td>0.35±0.08</td>
<td>0.07±0.04</td>
<td>0.29±0.03</td>
<td>0.12±0.01</td>
<td>NS</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Kidney (nmol/mg protein/min)</td>
<td>12.81±1.42</td>
<td>8.00±0.71</td>
<td>12.90±1.23</td>
<td>7.88±0.62</td>
<td>NS</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lung (nmol/mg protein/min)</td>
<td>0.81±0.07</td>
<td>0.56±0.08</td>
<td>0.41±0.05</td>
<td>0.32±0.03</td>
<td>&lt;0.0001</td>
<td>0.0118</td>
</tr>
</tbody>
</table>

*The animals were treated with a single dose of 300 mg/kg ONO-9902, and 24 h after the administration plasma and tissue samples were taken. NEP activity was determined by HPLC with an aid of synthetic peptide as described in Methods. Each value represents the mean±S.E.M. of 4–7 experiments. Two-way factorial ANOVA was used to determine whether plasma and tissue NEP activities were affected by CAL or treatment with ONO-9902. Differences in mean values for plasma and tissue NEP activities between ONO-9902 treated and untreated groups were tested by post-hoc comparison with Fisher’s PLSD method. NEP activity is expressed as the rate of 7-amino-4-methylcoumarin cleavage. NS, no significance.
were restored almost to sham levels and the decrease in AF tended to be restored by chronic treatment with ONO-9902. This drug had no significant effects on these hemodynamic variables of the Sham rats.

3.3. Effects of long-term treatment with ONO-9902 on changes in the plasma and left ventricular ANP, BNP and ET-1

Effects of long-term treatment with ONO-9902 on changes in the plasma ANP and BNP concentrations of rats with CAL and of Sham rats are shown in Fig. 4a and b, respectively. The plasma ANP and BNP concentrations were significantly increased in rats with CAL as compared with those of the Sham rats (two-way ANOVA). Long-term treatment with ONO-9902 significantly attenuated the increases in the plasma ANP concentration (176±14 to 124±3 pg/ml, \( P<0.0001 \), post-hoc test) and BNP concentration (81±4 to 53±3 pg/ml, \( P<0.0001 \), post-hoc test). Similarly, the LV ANP content was significantly increased by CAL. Long-term treatment with ONO-9902 prevented the increase in the LV ANP content (Table 6).

To determine whether long-term treatment with the NEP inhibitor might have affected the plasma ET-1 level and LV ET-1 content, we determined the effects of chronic treatment with ONO-9902 on changes in these parameters in rats with CAL and in Sham rats at the 6th week after the operation (Fig. 5). The plasma and LV ET-1 levels were significantly increased by CAL (two-way ANOVA). Chronic treatment with ONO-9902 significantly attenuated the increase in both levels.

4. Discussion

4.1. Short-term treatment with ONO-9902

In the present study, we found that a single administration of ONO-9902 resulted in the inhibition of the plasma and renal NEP activities, which was accompanied by a significant elevation of the plasma ANP level in rats with CAL. This is consistent with the effects of the NEP inhibitor ecadotril (sinorphan), a NEP inhibitor that is an orally active prodrug of (S)-thiorphan, on the plasma NEP activity and the ANP concentration in rats with heart failure produced by aortovenocaval (AV) fistula as reported by other investigators [19]. In the Sham rats in vivo treatment with ONO-9902 also diminished the plasma and tissue NEP activities but the plasma concentrations of ANP and BNP were not increased. It is considered that the inactivation of circulating ANP and BNP is attributed to clearance receptor-mediated internalization and/or enzymatic degradation of these peptides and that NEP plays a major role in the clearance of ANP when its plasma concentration of the animals with heart failure is high [10]. Therefore, it is conceivable that NEP inhibitors may

![Graph showing the effects of acute treatment with ONO-9902 on changes in the plasma ANP (a) and BNP (b) concentrations of rats with left coronary artery ligation (CAL) or in those of sham-operated rats (Sham) at the 1st week after the operation. Data are shown as the means±S.E.M. of 6–8 experiments. Numbers in parentheses indicate the numbers of animals. Two-way factorial ANOVA revealed that both CAL and ONO-9902 treatment affected the plasma ANP, but not the BNP concentration. * P<0.05 vs. ONO-9902 untreated Sham; # P<0.05 vs. CAL.](image-url)
Effects of long-term treatment with ONO-9902 on changes in body and organ weights in rats with left coronary artery ligation (CAL) and in sham-operated rats (Sham) at the 6th week after the operation

<table>
<thead>
<tr>
<th>Weights</th>
<th>Sham Untreated</th>
<th>Sham Treated</th>
<th>CAL Untreated</th>
<th>CAL Treated</th>
<th>P (ANOVA)</th>
<th>P (untreated vs. treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body (g)</td>
<td>329±4</td>
<td>332±4</td>
<td>286±4</td>
<td>285±4</td>
<td>&lt;0.0001</td>
<td>NS NS NS NS</td>
</tr>
<tr>
<td>Lung (mg)</td>
<td>995±12</td>
<td>963±16</td>
<td>2076±65</td>
<td>1768±96</td>
<td>&lt;0.0001</td>
<td>0.0112 0.0387 NS NS</td>
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<tr>
<td>Lung (mg)/body wt. (g)</td>
<td>3.50±0.68</td>
<td>2.98±0.44</td>
<td>7.28±0.25</td>
<td>6.3±0.34</td>
<td>&lt;0.0001</td>
<td>0.0031 NS NS NS</td>
</tr>
<tr>
<td>LV (mg)</td>
<td>592±11</td>
<td>593±8</td>
<td>540±26</td>
<td>519±13</td>
<td>0.0032</td>
<td>NS NS NS NS</td>
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<tr>
<td>LV (mg)/body wt. (g)</td>
<td>1.71±0.05</td>
<td>1.73±0.03</td>
<td>1.85±0.11</td>
<td>1.78±0.03</td>
<td>&lt;0.0001</td>
<td>NS NS NS NS</td>
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<td>RV (mg)</td>
<td>121±9</td>
<td>120±9</td>
<td>204±26</td>
<td>186±24</td>
<td>&lt;0.0001</td>
<td>NS NS NS NS</td>
</tr>
<tr>
<td>RV (mg)/body wt. (g)</td>
<td>0.35±0.03</td>
<td>0.35±0.02</td>
<td>0.71±0.10</td>
<td>0.64±0.09</td>
<td>&lt;0.0001</td>
<td>NS NS NS NS</td>
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</table>

* Each value represents the mean±S.E.M. of 6–8 experiments. Two-way factorial ANOVA was used to determine whether body and organ weights were affected by CAL or treatment with ONO-9902. Differences in mean values for body and organ weights between ONO-9902 treated and untreated groups were tested by post-hoc comparison with Fisher’s PLSD method. Abbreviations: LV, left ventricular; RV, right ventricular; NS, no significance.

Effects of long-term treatment with ONO-9902 on changes in heart rate (HR), mean arterial pressure (MAP), left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), \( +dP/dt \) max and \( -dP/dt \) max in rats with left coronary artery ligation (CAL) and in sham-operated rats (Sham) at the 6th week after the operation

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<th>Sham Untreated</th>
<th>Sham Treated</th>
<th>CAL Untreated</th>
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<th>P (ANOVA)</th>
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<tr>
<td>HR (beats/min)</td>
<td>385±9</td>
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<td>377±7</td>
<td>386±7</td>
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<td>MAP (mmHg)</td>
<td>99±4</td>
<td>93±3</td>
<td>92±4</td>
<td>100±4</td>
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<td>LVSP (mmHg)</td>
<td>127±5</td>
<td>119±6</td>
<td>111±3</td>
<td>115±4</td>
<td>NS NS NS NS</td>
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<td>LVEDP (mmHg)</td>
<td>2.16±0.22</td>
<td>4.54±1.04</td>
<td>26.64±1.40</td>
<td>21.47±1.22</td>
<td>&lt;0.0001 0.0089 0.0028</td>
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<td>( +dP/dt ) max (mmHg/s)</td>
<td>9342±564</td>
<td>8850±846</td>
<td>6000±339</td>
<td>6975±396</td>
<td>&lt;0.0001 NS NS NS</td>
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<tr>
<td>( -dP/dt ) max (mmHg/s)</td>
<td>9917±513</td>
<td>10 050±696</td>
<td>4183±311</td>
<td>5313±380</td>
<td>&lt;0.0001 NS NS NS</td>
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</tbody>
</table>

* Each value represents the mean±S.E.M. of 6–8 experiments. Two-way factorial ANOVA was used to determine whether hemodynamic parameters were affected by CAL or treatment with ONO-9902. Differences in mean values for HR, MAP, LVSP, LVEDP, \( +dP/dt \) max and \( -dP/dt \) max between ONO-9902 treated and untreated groups were tested by post-hoc comparison with Fisher’s PLSD method. NS, no significance.

Enhance the biological activity of endogenous ANP and thereby produce the favorable effect only when the plasma ANP level is high.

Like plasma ANP, plasma BNP is recognized as a sensitive marker for the degree of severity of CHF [21]. Our results showed that plasma BNP in CAL animals was not increased by the acute treatment with ONO-9902, whereas plasma ANP was. A similar result was reported in the case of acute treatment with ecdotril, a NEP inhibitor, in rats with heart failure induced by AV fistula [19]. One possibility for the difference in response to CHF is that the affinity of BNP to NEP may be less than that of ANP. In
Fig. 4. Bar graphs showing the effects of long-term treatment with ONO-9902 on changes in the plasma ANP (a) and BNP (b) concentrations of rats with left coronary artery ligation (CAL) or in those of sham-operated rats (Sham) at the 6th week after the operation. Data are shown as the means ± S.E.M. of 7–8 experiments. Numbers in parentheses indicate the numbers of animals. Two-way factorial ANOVA revealed that the plasma ANP and BNP concentration were affected by CAL and ONO-9902. *, P < 0.05 vs. ONO-9902 untreated Sham; #, P < 0.05 vs. CAL.

Table 6
Effects of a long-term treatment with ONO-9902 on left ventricular (LV) ANP content of rats with left coronary artery ligation (CAL) and of sham-operated rats (Sham) at the 6th week after the operation*

<table>
<thead>
<tr>
<th></th>
<th>LV ANP content (pg/g frozen tissue)</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>Treated</td>
</tr>
<tr>
<td>Sham</td>
<td>128.9 ± 16.2</td>
<td>202.9 ± 36.7</td>
</tr>
<tr>
<td>CAL</td>
<td>2748.7 ± 492.5</td>
<td>1051.6 ± 191.3</td>
</tr>
</tbody>
</table>

* Each value represents the mean ± S.E.M. of six experiments. Two-way factorial ANOVA was used to determine whether LV ANP content was affected by CAL or treatment with ONO-9902. Differences in mean values for LV ANP content between ONO-9902-treated and untreated groups were tested by post-hoc comparison with Fisher’s PLSD method.
* * P < 0.05 vs. ONO-9902-untreated CAL (post-hoc test).
addition, the degradation of rat BNP due to NEP has been shown to be less than that of rat ANP [10,22]. Such pharmacokinetic differences may contribute to the lack of ONO-9902-induced NEP inhibition leading to an increase in plasma BNP.

4.2. Long-term treatment with ONO-9902

The major finding in the present study was that long-term treatment with ONO-9902 appreciably attenuated the CHF-induced increases in LVEDP and lung/body weight. Furthermore, ONO-9902 treatment suppressed the CHF-induced increase in SVR and decrease in COI. These results suggest that chronic treatment with this NEP inhibitor may prevent the CHF-induced increases in both left ventricular pre- and after-load. Since this drug did not affect MAP, the reduction in SVR may secondarily improve COI in rats with CAL. To our knowledge, there are very few reports showing that long-term treatment with a NEP inhibitor improved cardiac function. Yoshida et al. [23] reported that long-term treatment with the NEP inhibitor candaxotril had no effect on the cardiac contractile function and plasma ANP level, though the renal NEP activity was inhibited by the treatment. We cannot explain the reason for the difference in these observations. One possibility is that the dose of candaxotril (10 mg/kg/day) may have been so low that the plasma ANP level could not reach the therapeutically effective levels in their model.

We found that the increases in plasma concentrations of ANP and BNP and LV content of ANP in rats with CAL were attenuated by chronic treatment with ONO-9902. Since ONO-9902 prevents the metabolic degradation of ANP and BNP, this fact seems to be discrepant with the enzymatic inhibitory action of ONO-9902. One explanation for this apparent contradiction is that long-term treatment with ONO-9902 might lead to desensitize NEP of the rat with CAL, resulting in an alteration of the inhibitory action of this drug on NEP. It has been reported that in rats with heart failure produced by AV fistula, long-term treatment with ecadotril elevated the plasma ANP concentration and improved the diminished renal function [19]. The latter finding suggests that an increase in the plasma concentration of ANP is a prerequisite for long-lasting improvement of hemodynamic function by NEP inhibitor. AV fistular model exhibited greater preload pressure, a strong stimulant for ANP synthesis, and thereby caused a larger increase in the plasma ANP unlike the CHF model following myocardial infarction. Thus, in the AV fistular model, the elevated preload pressure may be enough to augment ANP biosynthesis in the myocardium. In contrast, in rats with CHF following myocardial infarction, the reduction in preload pressure, as shown by LVEDP of the ONO-9902-treated rat with CAL, may account for the mechanism by which the amount of the compensatory biosynthesis of ANP and also probably BNP in the non-infarct myocardium was decreased. The decrease in synthesis of ANP and BNP seems to contribute to the reduction in these plasma concentrations. Moreover, plasma concentrations of these peptides are sensitive indices for the severity of heart failure as described above [21]. Therefore, we consider the reduction in the plasma ANP and BNP concentrations following chronic treatment with ONO-9902 to be as a result of the improvement of the pathophysiology of CHF.

The mechanism by which chronic treatment with ONO-9902 improved hemodynamic parameters of rats with CHF should be addressed. Since HR, cardiac contractility (LVSP and ±dP/dt max), and myocardial hypertrophy (heart weight) were not affected by ONO-9902 treatment, the direct action of NEP inhibitor on the heart is unlikely. Although ONO-9902 had no effect on MAP, it reduced LVEDP and SVR and increased COI. This implies that there may be vasodilation in both arterial and venous systems as similar to the consequence elicited by administration of a low dose of sodium nitroprusside [24]. Thus, the results suggest that chronic inhibition of NEP may improve pre- and after-load. A recent report demonstrated that 48-h infusion of ANP in patients with heart failure declined the mean pulmonary capillary wedge pressure and SVR secondary to diuresis and vasodilation [25]. This finding is consistent with the results in the present study.

In addition to ANP and BNP, ET-1, a potent vasoconstrictor peptide, also serves as a substrate for NEP. Indeed, acute treatment with the NEP inhibitor candaxotril increased the circulating plasma level of ET-1 in patients with heart failure [26]. Thus, it is possible to consider that long-term treatment with ONO-9902 increased the plasma ET-1 concentration and causes the detrimental effect in the setting of CHF. However, chronic treatment with ONO-9902 prevented the CHF-induced elevation of plasma and LV ET-1 levels. Thus, it is unlikely that chronic NEP inhibition elevates plasma ET-1 level and worsens the severity of heart failure.

In conclusion, long-term NEP inhibition by ONO-9902 exerted beneficial effects on hemodynamic and humoral factors probably by increasing the plasma concentration of ANP during the early period of administration to rats with CHF. This drug may offer a new and effective therapeutic approach to CHF.

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