Chronic Effect of Combined Treatment With Omapatrilat and Adrenomedullin on the Progression of Heart Failure in Rats

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Background: We and other investigators have reported that short- and long-term treatment with adrenomedullin has beneficial effects in heart failure. This study examined the effects of long-term treatment with a vasopeptidase inhibitor plus adrenomedullin in a model of heart failure in rats and assessed potential mechanisms of action.

Methods: Dahl salt-sensitive rats aged 11 weeks were randomly divided into three groups: an omapatrilat group, an omapatrilat plus adrenomedullin group, and an untreated group. The effects of these treatments were evaluated after 7 weeks of treatment.

Results: Omapatrilat monotherapy significantly improved left ventricular weight (LVW), blood pressure (BP), and central hemodynamics as compared with the untreated group. Omapatrilat decreased the gene expression levels of adrenomedullin and atrial natriuretic peptide (ANP) in the left ventricle. In addition, omapatrilat decreased mRNA levels of transforming growth factor-β (TGF-β), collagen I, collagen III, plasminogen activator inhibitor-1 (PAI-1), and intercellular adhesion molecule-1 (ICAM-1) in the left ventricle, and omapatrilat decreased perifibrosis score and myocyte area histologically. Omapatrilat plus adrenomedullin further improved LVW, central hemodynamics, and mRNA expression of TGF-β, collagen I, collagen III, PAI-1, and ICAM-1 without changing BP. Omapatrilat plus adrenomedullin further reduced mRNA levels of ANP and adrenomedullin without altering levels of ANP or adrenomedullin in plasma. Interestingly, omapatrilat slightly decreased mRNA levels of subunits of NADPH oxidase, whereas omapatrilat plus adrenomedullin further decreased these variables.

Conclusions: Our results suggest that combined treatment with adrenomedullin and omapatrilat may be a new strategy for the management of heart failure, acting partly by inhibition of the extracellular matrix gene, adhesion molecule, antifibrinolysis, and oxidative stress production. Am J Hypertens 2006;19:1039–1048 © 2006 American Journal of Hypertension, Ltd.

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Previous studies demonstrated that adrenomedullin (AM) infusion causes vasodilation, diuresis, and natriuresis and inhibits aldosterone secretion in healthy animals.1 Clinical studies have shown that plasma AM levels are related to the severity of heart failure.2,3 These findings suggest that AM has an important role in maintaining volume and pressure homeostasis in heart failure. Short-term treatment with AM improves central hemodynamics, renal function, and hormone levels in animal models of heart failure, as well as in patients with heart failure.4–7 In addition, long-term treatment with AM prevents the transition from left ventricular hypertrophy to heart failure in rats.8 Therefore, available evidence indicates that AM has an important pathophysiologic role in heart failure.

Neutral endopeptidases are localized in many tissues and cleave endogenous peptides,9 including AM. These
enzymes therefore play a key part in the degradation of AM. A previous study has shown that neutral endopeptidase inhibition potentiates the natriuretic actions of exogenous AM in anesthetized dogs. A combination of a neutral endopeptidase inhibitor and AM may be more therapeutically beneficial than either agent alone. Studies by Rademaker and co-workers have confirmed this hypothesis in sheep with heart failure given short-term treatment with AM plus a neutral endopeptidase inhibitor. Preliminary evidence thus suggests that combining AM with a neutral endopeptidase inhibitor would provide additional benefits in heart failure. Very recently we have shown that long-term treatment with omapatrilat and AM has better renoprotective effect than AM monotherapy in hypertensive rats. However, whether long-term treatment with AM plus a neutral endopeptidase inhibitor delays or prevents the development of heart failure remains unknown.

Vasopeptidase inhibitors are novel compounds that provide dual inhibition of angiotensin-converting enzyme (ACE) and neutral endopeptidases. In addition to suppressing angiotensin II production, vasopeptidase inhibitors inhibit the degradation of bradykinin, natriuretic peptides, AM, and substance P. Thus, vasopeptidase inhibitors may be more effective in heart failure than ACE inhibitors or neutral endopeptidase inhibitors alone.

This study was designed to test the hypothesis that long-term treatment with AM plus a vasopeptidase inhibitor is more effective than treatment with a vasopeptidase inhibitor alone in a model of heart failure in rats. A secondary objective was to investigate potential mechanisms of drug action. Adrenomedullin exerts as an antifibrotic factor in the heart in vitro. In addition, recent studies have shown the antioxidative effect of endogenous and exogenous administered AM. In oxidative stress, NADPH oxidase subunits play important roles in the certain pathophysiological condition. Therefore, we attempted to assess the potential mechanisms of its action.

Methods

All procedures were conducted in accordance with our institutional guidelines for animal research.

Experimental Animals and Protocols

Male inbred Dahl salt-sensitive (DS) rats (Eisai Co., Ltd., Tokyo, Japan) were fed a diet containing 8% NaCl (high salt) after the age of 6 weeks. At 11 weeks of age, the rats were randomly divided into three groups: an omapatrilat group, an omapatrilat plus AM group, and an untreated group. The omapatrilat group was given in an average dose of 35 mg/kg/d in the drinking water for 7 weeks. The AM was given for 7 weeks by means of an osmotic minipump (model 2 ML4, Alza Corporation, Palo Alto, CA), as previously reported. The minipump with filled with recombinant human AM dissolved in 0.9% saline in the omapatrilat plus AM group (500 ng/h per rat) and with 0.9% saline in the untreated group. Male Dahl salt-resistant (DR) rats, fed the same diet, served as control group.

Hemodynamic Measurements and Blood Sampling

All rats had their systolic blood pressure (BP) measured by the tail–cuff method before starting the high salt diet, and at 2-week intervals thereafter. After 7 weeks of treatment, mean arterial pressure (MAP), right ventricular systolic pressure (RVSP), right atrial pressure (RAP), and left ventricular end-diastolic pressure (LVEDP) were measured, as described previously. Then, 5 mL of blood was obtained as described previously. The right and left ventricle and atrium were isolated, weighed, frozen in liquid nitrogen, and stored at −80°C. Half of the left ventricle was postfixed in 10% neutral buffered formalin.

Recombinant Human Adrenomedullin

Human recombinant AM was kindly provided by Shionogi & Co., Ltd., Osaka, Japan. The production method of human recombinant AM has been described previously.

Hormonal Analysis

Plasma renin concentration (PRC), plasma aldosterone, atrial natriuretic peptide (ANP), cAMP, and cGMP concentration were measured by radioimmunoassay, as previously reported. Plasma human AM was measured using specific immunoradiometric assay (IRMA) kit (AM RIA SHIONOGI, Osaka, Japan). Rat total AM was also measured by IRMA systems as previously reported. Each IRMA system specifically recognizes rat or human AM and does not cross react with other AM.

Histopathologic Analysis

The perivascular fibrosis area and the perivascular fibrosis ratio (the ratio of the fibrosis area surrounding the vessel to the total vessel area) were calculated as previously reported. To evaluate the extent of cardiomyocyte hypertrophy, cross-sectional images of cardiomyocytes at the subendocardium and the epicardium were scanned with a computer analysis system, as previously reported.

Northern Blot Analysis

Northern blot analysis for the evaluation of AM and ANP mRNA expression was performed as described in detail in our previous report.

Quantification of Messenger RNA by Reverse Transcriptase–Polymerase Chain Reaction

All procedures used for mRNA extraction, cDNA synthesis, and polymerase chain reaction (PCR) have been described in detail previously. The numbers of PCR cycles...
for the 10 genes examined were as follows: transforming growth factor-β (TGF-β), 30; collagen I, 31; collagen III, 28; plasminogen activator inhibitor-1 (PAI-1), 31; intercellular adhesion molecule-1 (ICAM-1), 32; p40phox, 31; p22phox, 29; gp91phox, 33; p47phox, 32; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 22. Each species of mRNA was quantified with the following formula: amount of original template of each molecule/amount of original template of GAPDH.

**Statistical Analysis**

All values are expressed as means ± SD. Statistical comparisons among the four groups were carried out by ANOVA followed by Bonferroni’s test for multiple comparisons. *P* values of < .05 were considered to indicate statistical significance.

**Results**

**Physiologic Profiles**

The physiologic profiles of the four experimental groups are summarized in Table 1. Body weight (BW) was significantly lower in untreated DS rats than in DR rats. In contrast, the weights of the left ventricle (LV), right ventricle (RV), left atrium (LA), and right atrium (RA), and the lung weight/BW were higher in untreated DS rats than in DR rats. Long-term omapatrilat monotherapy in the DS rats significantly increased BW and decreased LV weight/BW, RV weight/BW, LA weight/BW, RA weight/BW, and lung weight/BW as compared with untreated DS rats. Chronic treatment with omapatrilat plus AM also improved these variables and further decreased LV weight/BW and lung weight/BW as compared with omapatrilat monotherapy.

**Central Hemodynamic Responses**

As shown in Fig. 1A, hypertension progressively developed in DS rats fed a high salt diet from 6 weeks of age. Long-term treatment with omapatrilat alone and omapatrilat plus AM similarly reduced systolic BP in DS rats at 13, 15, and 17 weeks. As shown in Fig. 1B to F, untreated DS rats at 18 weeks were characterized by higher RVSP, RAP, LVEDP, and MAP as compared with DR rats. Long-term omapatrilat monotherapy significantly reduced RVSP, RAP, LVEDP, and MAP. Combined treatment with omapatrilat and AM was more effective in reducing RVSP, RAP, and LVEDP than omapatrilat monotherapy, whereas MAP was similar with both of these treatments.

**mRNA Expression Levels of AM and ANP in the LV, and Levels of Neurohumoral Factors**

As shown in Fig. 2A,B, the mRNA expression levels of ANP and AM were increased in DS rats as compared with DR rats. Omapatrilat monotherapy significantly decreased the mRNA expression of ANP and AM as compared with untreated DS rats. Combined therapy with omapatrilat and AM further decreased the mRNA expression of ANP and AM as compared with omapatrilat-treated DS rats. However, expression levels were still higher than those in DR rats.

As shown in Fig. 2C to H, untreated DS rats had higher plasma ANP, rat endogenous AM, cGMP, cAMP, and aldosterone levels, as well as higher PRC than DR rats. Omapatrilat monotherapy did not change plasma ANP, rat AM, cGMP, cAMP, or aldosterone levels. Omapatrilat plus AM did not alter the plasma ANP, rat AM, cGMP, or cAMP levels, but decreased the plasma aldosterone level as compared with untreated DS rats. Thus, there were obvious differences between mRNA levels and plasma levels of AM and ANP, because of inhibition of neutral endopeptidase. In contrast, omapatrilat monotherapy increased PRC as compared with untreated DS rats, whereas omapatrilat plus AM decreased PRC as compared with omapatrilat monotherapy, although PRC remained higher than that in DR rats. Human AM, which was infused only in the omapatrilat plus AM group, was detected in only DS rats given omapatrilat plus AM (3.3 ± 1.2 pmol/L, *n* = 1041).
FIG. 1. (A) Time course of systolic BP (SBP) in four groups. DS rats were randomly divided into three groups: untreated, treated with omapatrilat (Oma), or treated with omapatrilat plus AM from 11 weeks of age. (B to F) Central hemodynamics in DR and DS rats at 18 weeks. Right ventricular systolic pressure (RVSP) (B), right atrial pressure (RAP) (C), left ventricular end-diastolic pressure (LVEDP) (D), heart rate (HR) (E), and mean arterial pressure (MAP) (F). *P < .05 v DR, **P < .01 v DR, †P < .05 v DS, ††P < .01 v DS, #P < .05 v DS + omapatrilat.

*P < 0.01 vs DR
†P < 0.05 vs DS

omapatrilat or omapatrilat + AM
8% NaCl
FIG. 2. Myocardial gene expression of ANP and AM in four groups. Left ventricular (LV) ANP mRNA level (A) and AM mRNA level (B). Top of (A) and (B) Representative autoradiograms of LV ANP, AM, and GAPDH mRNA bands. (C to H). Plasma neurohormonal factors in four groups. Plasma ANP level (C), plasma rat endogenous AM level (Rat AM) (D), plasma cGMP level (E), plasma cAMP level (F), plasma aldosterone level (G), and plasma renin concentration (PRC) (H). *P < .05 v DR, **P < .01 v DR, †P < .05 v DS, ††P < .01 v DS, †††P < .05 v DS + omapatrilat.
12). These concentrations were higher than those in our previous long-term AM monotherapy in DS rats\textsuperscript{17} (2.1±1.4 pmol/L, \( n = 12, P \leq 0.05 \)) and malignant hypertensive rats\textsuperscript{18} (0.7±0.5 pmol/L, \( n = 8, P \leq 0.001 \)).

**Histologic Findings in the LV and mRNA Expressions of PAI-1 and ICAM-1**

As shown in Fig. 3, the fibrosis area (Fig. 3E) and perivascular fibrosis score (Fig. 3F) were significantly greater in untreated DS rats than in DR rats. Omapatrilat monotherapy significantly decreased the fibrosis area and perivascular fibrosis score as compared with untreated DS rats. Treatment with omapatrilat plus AM similarly decreased the fibrous area and further decreased the perivascular fibrosis score, although these variables remained higher than those in DR rats. Myocytes area at subendocardium (Fig. 3G) and subepicardium (Fig. 3H) were also significantly greater in untreated DS rats than in DR rats. Omapatrilat monotherapy significantly decreased myocytes area at the subendocardium and subepicardium as compared with untreated DS rats. Both therapy similarly decreased the fibrous area and further decreased the perivascular fibrosis score, although these variables remained higher than those in DR rats. To examine the mechanism by which combined treatment with omapatrilat and AM improved the vascular remodeling, we measured mRNA expression levels of PAI-1 and ICAM-1. As shown in Fig. 3IJ, the mRNA expression levels of PAI-1 and ICAM-1 in the LV were increased in untreated DS rats as compared with DR rats. Omapatrilat treatment significantly decreased the mRNA expression levels of PAI-1 and ICAM-1, whereas treatment with omapatrilat plus AM further decreased the mRNA expression of PAI-1 and ICAM-1.

**Gene Expression Levels of TGF-\( \beta \), Collagen I, and Collagen III in the LV**

As shown in Fig. 4A to C, the mRNA expression levels of TGF-\( \beta \) (Fig. 4A), collagen I (Fig. 4B), and collagen III (Fig. 4C) were increased in untreated DS rats as compared with DR rats. Omapatrilat monotherapy significantly decreased the mRNA expression levels of TGF-\( \beta \), collagen I, and collagen III in the LV. Combined treatment with omapatrilat and AM further decreased the mRNA expression levels of these variables.

**mRNA Expression Levels of NADPH Oxidase Subunits**

As shown in Fig. 4D to F, the mRNA expression levels of p22phox (Fig. 4D), gp91phox (Fig. 4E), p40phox (Fig. 4F), p47phox (Fig. 4G), and p67phox (Fig. 4H) in the LV were increased in untreated DS rats as compared with DR rats. Both treatments similarly reduced the mRNA expression of p22phox. Whereas omapatrilat treatment slightly decreased the mRNA expression levels of p40phox, p47phox, p67phox, and gp91phox in the LV, treatment with omapatrilat plus AM further decreased the mRNA expression of these NADPH subunits. However, the expression levels of p40phox, p47phox, and gp91phox remained higher than those in DR rats.

**Discussion**

In the present study, the improvements in central hemodynamic variables such as LVEDP, RVSP, and RAP were greater with AM plus omapatrilat than with omapatrilat alone. The mechanism underlying the beneficial hemodynamic effects of long-term treatment with AM may differ from that responsible for the acute effects of AM. Because concurrent administration of AM did not alter BP as measured by the tail–cuff method and direct measurement, the beneficial hemodynamic effects of combined therapy may be due to the direct cardioprotective effects of AM, rather than direct effects on systemic and pulmonary arterial tone. We previously reported that AM monotherapy attenuated the progression of heart failure in DS rats without changing MAF.\textsuperscript{8} Although we cannot simply compare the progression that was only observed in omapatrilat monotherapy, the inhibitory effects of omapatrilat for heart failure was greater than AM monotherapy. In addition, the reduction in LV weight, improvement in histologic findings, and decrease in the expression of several remodeling-related genes were greater with AM plus omapatrilat than with omapatrilat alone. Previous studies have demonstrated that AM gene and peptide are strongly expressed in cardiac myocytes and fibroblasts and have suggested that endogenous cardiac AM plays important roles in cardiac hypertrophy and remodeling by acting as an autocrine or paracrine factor.\textsuperscript{14,24} Neutral endopeptidase is the major enzyme responsible for the degradation of AM.\textsuperscript{4,10} In the rat, myocytes have shown intense, positive neutral endopeptidase staining along the plasma membrane and in the cytoplasm.\textsuperscript{9} These results indicate that the cardioprotective effects of AM are in part ascribed to the inhibition of neutral endopeptidase and consequent augmentation of endogenous plasma and cardiac AM levels.

Vasopeptidase inhibition is a new concept in cardiovascular therapy based on the simultaneous inhibition of two key enzymes, ACE and neutral endopeptidase, both involved in various ways in the regulation of cardiovascular function.\textsuperscript{13} Neutral endopeptidases affect the metabolism of several vasoactive peptides including ANP and AM and regulate their clearance from the circulation.\textsuperscript{25} Interestingly, dissociation between plasma levels and gene expression levels of AM and ANP was observed. The reduction of mRNA levels of AM and ANP are due to the cardioprotective effect of omapatrilat and omapatrilat plus AM therapy. Whereas the higher plasma AM and ANP levels, despite the lower gene expression levels, provide evidence of inhibition of neutral endopeptidase. This notion is further supported by the findings of higher plasma cGMP, a second messenger of ANP, and higher plasma cAMP, a second messenger of AM, in the omapatrilat and
FIG. 3. Histologic findings of coronary arterioles and myocyte areas at the subendocardium and epicardium in four groups. (Top) Representative coronary arterioles (A–D). (Bottom) Perivascular fibrosis area (E), perivascular fibrosis ratio (F), myocyte areas at two depths, subendocardium (G) and subepicardium (H). Data are expressed as means ± SD. *P < .05 vs DR, **P < .01 vs DR, †P < .05 vs DS, ††P < .01 vs DS, #P < .05 vs DS + omapatrilat, #†P < .01 vs DS + omapatrilat.
omapatrilat plus AM groups. Thus, increased levels of ANP and AM may contribute in part to the beneficial effects of omapatrilat. In the present study, we infused human AM at a rate of 500 ng/h. The plasma level of human AM was higher than that in previous studies using the same infusion rate in the same rat model\(^\text{17}\) and in the malignant hypertensive rat model.\(^\text{18}\) Thus, neutral endopeptidase inhibition by omapatrilat apparently elevated plasma levels of not only rat endogenous AM, but also of exogenously administered human AM. The DS rats had higher plasma aldosterone levels than did DR rats; however, omapatrilat alone did not change it. The effect of omapatrilat on plasma aldosterone levels seems to be controversial. Omapatrilat significantly decreased plasma aldosterone levels in sheep with heart failure induced by pacing,\(^\text{26}\) whereas omapatrilat did not change it in dog with heart failure.\(^\text{27}\) Thus, different study protocol, dose of omapatrilat, animal species, or type of heart failure, and other variables may explain these different results. In addition, compared with these studies, we administered omapatrilat for a longer period. Therefore, we could not exclude the possibility that the aldosterone escape phenomenon might be involved in the current results. In contrast, a decrease in plasma aldosterone was observed only with AM plus omapatrilat. This finding agrees with the results of previous studies showing that AM inhibits aldosterone secretion in hypertension,\(^\text{12,17}\) and heart failure.\(^\text{7,8}\) Thus, inhibitory effect of combined therapy on aldosterone secretion is possibly due to the direct effect of AM.\(^\text{1}\) In contrast, omapatrilat monotherapy stimulated PRC in our study by inhibiting ACE activity. Combined treatment with AM and omapatrilat significantly decreased the PRC as compared with omapatrilat alone. Adrenomedullin might decrease basal renin levels by improving heart failure and renal impairment.\(^\text{8,17,18}\)

In the process of vascular remodeling, many genes relating to extracellular matrix, adhesion molecule, and fibrinolysis are involved.\(^\text{28,29}\) In the present study, omapatrilat decreased the fibrosis area, perivascular fibrosis, and mRNA expression levels of TGF-β, collagen I, collagen III, PAI-1, and ICAM-1 in the LV. In addition, combined treatment with AM and omapatrilat further decreased perivascular fibrosis and mRNA expression levels of TGF-β and collagens I and III in the LV. The reduction of mRNA expressions, such as TGF-β and collagens I and III, is consistent with the previous findings in deoxycorticosterone acetate salt-treated rats.\(^\text{30}\) Previous studies demonstrated that AM inhibits collagen synthesis in cardiac fibroblasts in vitro through the cAMP signaling pathway.\(^\text{14}\) In addition, human AM gene delivery inhibits cardiac fibrosis in several rat models of cardiac hypertrophy, remodeling, and myocardial infarction.\(^\text{3,19}\) We recently showed that endogenous AM acts as an autocrine antifibrinotic factor.\(^\text{14}\) In addition, previous studies showed that AM has antivascular remodeling action in vivo.\(^\text{31}\) Adrenomedullin alters the gene expression of the adhesion molecule and coagulation-relating molecule in vascular endothelial cells. In the present study, vascular remodeling and perivascular fibrosis were associated with the increased gene expressions of PAI-1 and ICAM-1 in DS, and combined therapy decreased them. Thus, our results confirm and extend these previous findings by demonstrating for the first time that concurrent treatment with AM and omapatrilat inhibits cardiac perivascular fibrosis mediated by inhibition of the gene expression of extracellular matrix, adhesion molecule, and antifibrinolysis.
Although the precise mechanism underlying the beneficial effects of AM remains unclear, a growing body of evidence suggests that inhibition of oxidative stress is involved. A previous study showed that AM inhibited the generation of oxygen radical metabolites in cultured mesangial cells and macrophages. Furthermore, recent studies have shown that reactive oxidative stress and the gene expression of NADPH oxidase subunits are higher in AM heterozygous knockout mice than in wild-type mice in various models of cardiovascular disease, indicating that endogenous AM exerts an antioxidative factor. In addition, treatment with AM has been shown to reduce reactive oxidative stress and improve these pathologic conditions as well as antioxidative drugs. These results suggest that AM exerts organ protective effects, in part, by inhibiting the production of oxidative stress. In the present study, combined treatment with AM and omapatrilat was more effective in reducing the mRNA expression of NADPH subunits such as gp91phox, p40phox, p47phox, and p67phox in the LV. Thus, treatment with AM plus omapatrilat may be more effective than omapatrilat alone, partly because of inhibition of oxidative stress.

In clinical settings, omapatrilat has been shown to be effective in the treatment of patients with hypertension and heart failure. However, omapatrilat increased the incidence of angioedema, which is a rare but potentially life-threatening side effect. Therefore, the clinical use of a vasopeptidase inhibitor may be limited. To determine the risk/benefit ratio of a vasopeptidase inhibitor in clinical use, further studies are necessary.

In conclusion, our study demonstrates for the first time that long-term treatment with omapatrilat plus AM has beneficial hemodynamic, plasma neurohormonal, myocardial biochemical, and cardiac functional effects, resulting in the improvement of heart failure in DS rats. Our findings indicate that long-term treatment with a vasopeptidase inhibitor plus AM may be a new therapeutic approach to the management of heart failure.

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References


