Cardiac and renal effects of omapatrilat, a vasopeptidase inhibitor, in rats with experimental congestive heart failure

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Abassi, Zaid A., Ali Yahia, Samar Zeid, Tony Karram, Eliahu Golomb, Joseph Winaver, and Aaron Hoffman. Cardiac and renal effects of omapatrilat, a vasopeptidase inhibitor, in rats with experimental congestive heart failure. Am J Physiol Heart Circ Physiol 288: H722–H728, 2005. First published October 21, 2004; doi:10.1152/ajpheart.00737.2004.—Omapatrilat (OMP) is a novel mixed inhibitor of angiotensin-converting enzyme (ACE) and neutral endopeptidase 24.11 (NEP), the enzyme that metabolizes natriuretic peptides. Congestive heart failure (CHF) is characterized by excessive sodium retention, attributed to both an excessive effect of angiotensin II and diminished responsiveness to natriuretic peptides. In this study, we examined the acute and chronic renal and cardiac effects of OMP in rats with compensated [urinary sodium excretion (UNaV) > 1,200 μeq/day] and decompensated (UNaV < 100 μeq/day) CHF, induced by a surgical aortocaval fistula (ACF). Bolus injection of OMP (10 mg/kg) to sham controls produced significant diuretic and natriuretic responses [UNaV increased from 0.67 ± 0.19 to 3.27 ± 1.35 μeq/min, P < 0.05; fractional sodium excretion (FENa) increased from 0.23 ± 0.06 to 0.95 ± 0.34%, P < 0.01] despite a significant decline in blood pressure (BP). Rats with compensated CHF displayed blunted diuresis and natriuresis to this dose of OMP but a significant decrease in BP. However, in rats with decompensated CHF, OMP induced significant natriuresis (FENa increased from 0.18 ± 0.15 to 0.82 ± 0.26%, P < 0.05) despite a further decrease in BP (from 90 ± 9 to 71 ± 6 mmHg, P < 0.01). Two weeks after ACF, the heart/body weight ratio was significantly greater in rats with CHF than controls (0.51 ± 0.026 vs. 0.30 ± 0.004%, P < 0.0001), and UNaV was significantly lower. Immediate or late (1 or 6 days after ACF) OMP treatment in the drinking water (140 mg/l) reduced cardiac hypertrophy to 0.41–0.43% (P < 0.01) and induced natriuresis. These results suggest that OMP improves both sodium balance and cardiac remodeling and might be advantageous to ACE inhibitors for the treatment of decompensated CHF.

cardiac hypertrophy; renal function; rat; angiotensin-converting enzyme inhibitor; neutral endopeptidase inhibitor

INCREASED ACTIVITY of the renin-angiotensin-aldosterone system (RAAS) is commonly found in advanced stages of congestive heart failure (CHF) (4, 12, 14, 35). Clinical and experimental data provide strong evidence that this axis is involved in the pathogenesis of the cardiac, renal, and vascular changes of CHF and hypertension (2, 4, 7, 12, 14, 35, 37). Moreover, inhibitors of angiotensin-converting enzyme (ACE) have been found to be highly beneficial in the treatment of patients with CHF (2, 4, 31a, 31b, 37). These agents are thought to exert their favorable effects through both afterload and preload reduction due to their vasodilating and diuretic and natriuretic effects (2, 7, 31a, 31b, 37).

The natriuretic peptides (NPs), i.e., atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), are peptides of predominantly cardiac origin with vasodilatory and natriuretic properties. Their circulating levels are elevated in CHF (28). It is thought that NPs play a counterregulatory role to the increased activity of the vasoconstrictor neurohumoral systems in CHF (28). However, the natriuretic and vasodilatory actions of NPs are significantly attenuated in CHF, particularly in advanced stages of the disease (2, 37). Angiotensin II has been implicated in the attenuated responsiveness to NPs (2, 7, 37). Besides clearance receptors (NPR-C), NPs are removed by neutral endopeptidase 24.11 (NEP), a ubiquitous enzyme found in the vasculature, kidney, and heart (6, 8). Previous studies have shown that pharmacological inhibition of NEP protects NPs from inactivation and potentiates their natriuretic and hypotensive effects. In human heart failure, NEP inhibition was reported to reduce cardiac filling pressure and inhibit the RAAS (6, 8).

Omapatrilat (OMP) is a novel, highly selective inhibitor of both ACE and NEP (6, 8). These dual inhibitory actions offer potential hemodynamic and neurohumoral advantages over the inhibition of each enzyme alone. Recent results in heart failure suggest beneficial hemodynamic and renal effects mediated by the synergistic ACE and NEP inhibition offered by this drug in both experimental (5, 17, 33) and clinical (15, 18, 19, 27) CHF. These effects include increased cardiac output and attenuated cardiac hypertrophy in both mild and severe experimental heart failure induced by pacing (33) or coronary ligation (5, 17). The Inhibition of Metalloproteinaze in a Randomized Exercise and Symptoms Study in Heart Failure study reported a greater improvement in New York Heart Association (NYHA) class and reduction in combined mortality/hospitalization end points for patients with systolic heart failure receiving OMP compared with ACE inhibitor alone (27). Interestingly, when compared with the ACE inhibitor lisinopril in this trial, the one parameter that best differentiated OMP from lisinopril was renal function. In particular, renal function was preserved to a greater extent with OMP compared with that obtained by the ACE inhibitor. Such renoprotection was also observed in experimental CHF, wherein acute vasopeptidase inhibition (VPI), but not ACE inhibition, increased the glomerular filtration rate (GFR) (8). However, in some

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studies, NEP and ACE inhibition failed to improve CHF signs or symptoms (11, 21, 23). This discrepancy may stem from differences in the severity of CHF, and particularly in the resulting renal function, in the various studies.

Previous studies from our laboratory have shown that the experimental model of rats with an aortocaval fistula (ACF) closely mimics the neurohumoral, renal, and cardiac manifestations of patients with severe CHF (2, 7, 37). These include a marked degree of cardiac hypertrophy and dilatation, increased activity of the vasoconstrictor RAAS and sympathetic nervous system as well as the NPs system, and a marked decrease in renal perfusion with sodium retention by the kidney (4, 12, 14, 35, 37). Furthermore, this model offers a unique feature to study the renal manifestations of CHF by the ability to subdivide animals with ACF into rats with compensated CHF and decompensated CHF on the basis of their daily sodium excretion (1, 2, 7, 37). The latter subgroup is characterized by a marked neurohumoral activation, avid renal sodium retention, and edema formation, leading to death from pulmonary edema, whereas the former group maintains a normal sodium balance. The RAAS is a major determinant of this distinction between compensated and decompensated CHF (1, 2, 7, 37).

The present study was designed to examine the acute and chronic effects of OMP on renal function and cardiac hypertrophy in compensated and decompensated volume overload CHF induced by the creation of ACF and to assess whether it offers a more advantageous approach to enalapril in the treatment of cardiac hypertrophy.

**MATERIALS AND METHODS**

All experiments were conducted according to the guidelines of the Animal Use and Care Committee, Technion. Studies were conducted on male Wistar rats of local strain weighing 260–350 g. The animals were kept in individual metabolic cages, in a controlled-temperature room, and were fed standard rat chow containing 0.5% NaCl and tap water ad libitum.

**Experimental Model**

An ACF was surgically created between the abdominal aorta and inferior vena cava according to the method originally described by Stumpe et al. (31) and adapted in our laboratory (1, 2, 7, 37). Briefly, a midline abdominal incision was performed under pentobarbital sodium (Nembutal) anesthesia to expose the vena cava and abdominal aorta distal to the origin of the renal arteries. A longitudinal incision was performed in the outer wall of the vena cava. The common wall between the aorta and vena cava was grasped through the incision under binocular magnification, and a fistula (1.0–1.2 mm outer diameter) was created between the two vessels. The opening of the outer wall of the vena cava was closed with a continuous suture (7-0 prolene nonabsorbable suture, Ethicon). After the surgical procedure, the animals were allowed to recover and then returned to the metabolic cages for daily monitoring of urine output and sodium excretion. A matched group of sham-operated rats served as controls. Six to seven days after the operation, rats with ACF were divided into two subgroups according to their daily absolute rate of sodium excretion (UNaV) (37): rats with decompensated CHF (UNaV > 100 μeq/24 h) and rats with compensated CHF (UNaV > 1,200 μeq/24 h).

![Fig. 1. Effects of omapatrilat (OMP; 10 mg/kg iv) administration on urinary flow rate (V; A), glomerular filtration rate (GFR; B), absolute urinary sodium excretion (UNaV; C), and fractional sodium excretion (FENa; D) in sham-operated rats (n = 7) and compensated (n = 8) and decompensated (n = 6) rats with congestive heart failure (CHF). *P < 0.05 vs. baseline (B); #P < 0.05 vs. sham controls.](http://ajpheart.physiology.org/)

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Acute Studies

Effects of OMP on renal function. These studies were designed to evaluate the acute effects of OMP on urine flow, UNaV, and GFR in rats with compensated (n = 8) and decompensated (n = 6) rats with CHF. *P < 0.05 vs. sham controls; #P < 0.05 vs. sham controls; P < 0.05 vs. rats with compensated CHF.

Chronic Studies

Effects of OMP or enalapril on renal function and cardiac hypertrophy. These studies were designed to evaluate the acute effects of OMP on urine flow, UNaV, and GFR in rats with compensated (n = 8) and decompensated (n = 6) CHF compared with sham-operated controls (n = 7). Rats were anesthetized with Inactin (100 mg/kg ip) and underwent a tracheostomy. Polyethylene catheters (PE-50) were inserted in the left carotid artery for measurements of mean arterial blood pressure (MAP), the jugular vein for infusion of various solutions, and in the urine bladder for urine collections. A solution of 0.9% saline containing 2% inulin was infused intravenously throughout the experiment at a rate of 1.0% body weight/h. Urine was collected, and blood pressure was recorded throughout the experiment. After two baseline periods of 30 min each, a bolus of OMP (10 mg/kg) was administered intravenously, followed by four additional clearance collections. Blood samples were obtained between each two clearance periods. Plasma was separated and kept at 4°C until assayed for inulin and electrolytes.

Chemical analysis. Concentrations of inulin in plasma and urine were measured by the anthrone method. Inulin clearance defined GFR. Urinary and plasma sodium concentrations were determined by flame photometry (model 943, Instrumentation Laboratories). cGMP concentration in urine was determined by radioimmunoassay (IBL; Hamburg, Germany).

Statistical Analysis

One-way ANOVA was used for group comparisons, and repeated-measures ANOVA was used for comparison of treatment values with baseline value in each group. Dunnett’s test was used for postANOVA evaluation. For comparison of the graphs representing control and experimental groups, two-way ANOVA was used. A value of P < 0.05 was considered significant. Data are expressed as means ± SE.

RESULTS

Acute Studies

Figure 1 summarizes the renal and systemic effects of acute administration of OMP to sham-operated controls and rats with compensated or decompensated CHF. Baseline values of sham-operated rats were as follows: urinary flow rate (V), 10.0 ± 2.0 l/min; UNaV, 0.67 ± 0.19 eq/min; GFR, 2.11 ± 0.13 ml/min; fraction sodium excretion (FENa), 0.23 ± 0.06%; and MAP, 120.7 ± 3.0 mmHg. GFR and MAP were lower in compensated rats compared with sham-operated ones (GFR, 1.49 ± 0.37 ml/min; MAP 96.7 ± 4.9 mmHg), whereas the other parameters were comparable with those of sham controls (V, 12.5 ± 3.0 l/min; UNaV, 0.65 ± 0.26 eq/min; and FENa.
0.39 ± 0.13%). These values were lower in rats with decompen-sated CHF [V, 5.6 ± 1.0 µl/min; UNaV, 0.046 ± 0.025 µeq/min (P < 0.05); GFR, 0.74 ± 0.08 ml/min (P < 0.05); FENA, 0.18 ± 0.15%; MAP, 89.8 ± 8.8 mmHg]. Administration of OMP to sham-operated rats significantly increased V, UNaV, and FENA without affecting GFR. These effects occurred despite a significant fall in MAP throughout the experiment (Fig. 2). The diuretic and natriuretic responses to OMP were blunted in rats with compensated CHF. However, a gradual insignificant decline in GFR and a significant decrease in MAP after OMP injection were observed in this subgroup of rats with ACF (Fig. 2). When the same dose of OMP was administered to rats with compensated CHF, it induced significant increases in urinary flow (V increased 2-fold, P < 0.05) and sodium excretion (UNaV increased 2-fold, P < 0.05, and FENA increased 4- to 5-fold, P < 0.05) 90–120 min after the treatment despite a significant fall in MAP throughout the experiment (Fig. 2). In contrast to compensated rats, GFR was unchanged in decompensated rats after OMP administration (Fig. 1). The effects of OMP on urinary excretion of cGMP (UcGMPV) in the three groups of rats are summarized in Fig. 3. Basal UcGMPV was significantly higher in rats with either compensated (P < 0.05) and decompensated CHF than controls (P < 0.05) (2.33 ± 0.4 pmol/min in controls vs. 15.4 ± 4.8 and 7.1 ± 1.5 pmol/min in compensated and decompensated rats, respectively). This trend became more obvious when values were corrected for GFR, i.e., UcGMPV/GFR (1.03 ± 0.15 pmol/ml in controls vs. 10.70 ± 1.75 and 8.4 ± 1.15 pmol/ml in compensated and decompensated rats, respectively). In response to OMP, UcGMPV did not significantly increase in sham controls (Fig. 3). These observations persist when values were corrected to GFR (Fig. 3). In contrast, administration of OMP to rats with ACF produced gradual increases in UcGMPV, which reached statistical significance in rats with decompensated CHF 90 and 120 min after drug administration (Fig. 3). Correction of the values for GFR strengthened these trends (Fig. 3).

**Chronic Studies**

**Effects of OMP on cumulative sodium excretion.** The effects of chronic early or late treatment with OMP on urine volume and cumulative sodium excretion in rats with ACF are summarized in Fig. 4. Figure 4A shows the cumulative sodium
excretion in sham controls and rats with ACF treated with OMP. Cumulative sodium excretion was lower in CHF rats compared with sham-operated controls (P < 0.0001, two-way ANOVA). Chronic early OMP administration to rats with ACF restored sodium excretion to the same extent as observed in sham controls. The natriuretic effect of OMP was evident from the third day of treatment. Likewise, when OMP was given 6 days after the placement of ACF (late treatment), it caused a significant increase in sodium excretion but took a longer period (~4 days) to produce this effect. Figure 4B depicts the effects of OMP on urinary flow in these experimental groups. Early treatment with OMP induced a significant (P < 0.0001) diuretic response (~2-fold increase in urinary volume) in rats with ACF (Fig. 4B). This effect started on the first day of treatment and was notable in the second week of treatment. Late treatment with OMP produced a significant (P < 0.0001, two-way ANOVA) diuresis, although it was less profound than that induced by early treatment (Fig. 4B).

Effects of OMP on cardiac hypertrophy. Figure 5 shows the effects of treatment with OMP (early and late treatment protocols) on the heart weight-to-body weight ratio (HW/BW) in rats with ACF. HW/BW, an index of cardiac hypertrophy, increased from 0.30 ± 0.004% in sham-operated rats to 0.51 ± 0.026% in rats with CHF (P < 0.0001). Interestingly, early or late treatments with OMP were comparably and highly beneficial in reducing cardiac hypertrophy. HW/BW decreased after early or late treatment with OMP to 0.43 ± 0.02% and 0.41 ± 0.017%, respectively (P < 0.01 vs. untreated CHF rats, respectively). Similarly, late or early treatments with enalapril reduced HW/BW to 0.44 ± 0.023% and 0.44 ± 0.017%, respectively (P < 0.01 vs. untreated CHF rats for both). Thus early or late administration of either OMP or enalapril reduced the volume overload-induced cardiac hypertrophy in rats with ACF.

DISCUSSION

The present study demonstrates that acute administration of OMP to sham controls produced significant diuretic and natriuretic responses along with a remarkable decline in blood pressure. Rats with compensated CHF displayed blunted diuretic and natriuretic responses to acutely injected OMP, whereas the hypotensive effect of this drug was preserved. In contrast, OMP was specifically effective in rats with decompensated CHF, where it significantly increased urine flow and sodium excretion in association with a significant increase in UNaV. The stimulatory effects of OMP on renal excretory function in animals with decompensated CHF occurred despite a significant decline in MAP after drug administration. The natriuretic effect of OMP in decompensated CHF, which took place despite a remarkable fall in MAP, demonstrates that the blunted renal response to OMP in rats with compensated CHF is not due to the hypotensive action of the drug. Chronic early or late administration of OMP to rats with ACF provoked natriuresis and, to a lesser extent, diuresis. In addition, early or late treatment with either OMP or enalapril significantly and comparably reduced cardiac hypertrophy in rats with CHF.

The findings in this study support the concept that dual simultaneous inhibition of ACE and NEP may be used as an effective approach to counteract the adverse actions of RAAS and to restore, at least in part, the beneficial actions of NPs on the kidney in severe heart failure. In addition, they provide further evidence that the RAAS plays a major role in the pathogenesis of cardiac hypertrophy in CHF and that blockade of this system by vasopeptidase (VPIs) may be used as an additional or alternative therapy to the well-characterized ACE inhibitors.

Our experimental model, rats with ACF, is characterized by several features that closely mimic the pathophysiological consequences of CHF in patients. These include the characteristic neurohumoral activation of heart failure a decrease in renal perfusion/filtration (2, 29, 32, 37) and either the development of progressive sodium retention (decompensated CHF) or compensation and return to nearly normal sodium balance (compensated CHF) (1, 37). Previously, we have shown that RAAS is activated in those animals with severe sodium retention but not in those with normal sodium balance (1, 2, 37). Several studies in these rats (1, 7, 37) and in a similar model in dogs (34) have suggested that the deranged renal hemodynamics and augmented tubular sodium retention in CHF are highly dependent on activation of the RAAS. In agreement with this concept, acute administration of OMP into rats with decompensated CHF resulted in significant natriuresis, whereas administration of the same dose of OMP to animals with compensated CHF did not significantly alter UNaV. Likewise, the stimulatory action of OMP on GFR in decompensated CHF but not in rats with compensated CHF may contribute to the enhanced excretory effect of OMP in decompensated CHF.

The findings of the present study demonstrate that early or late chronic treatment with OMP resulted in marked natriuresis. These results are in line with some reports that demonstrated a greater natriuretic response of VPI in experimental CHF (9, 20, 32) compared with other conventional agents, such as ACE inhibitors. Administration of OMP into dogs with experimental heart failure induced by ventricular pacing pro-
duced a more profound natriuretic response compared with fosinoprilat (32). Furthermore, Burrell et al. (9) have shown that 4-wk treatment with S21402 to rats with experimental CHF due to myocardial infarction resulted in significant natriuresis, exceeding that observed with either NEP-I or captopril. Interestingly, in the Inhibition of Metalloproteinase in a Randomized Exercise and Symptoms Study in Heart Failure trial (27) in clinical symptomatic CHF, OMP was superior to lisinopril in reducing a combined end point of worsening heart failure and hospitalization compared with ACE inhibitors alone. It should be emphasized that the only significant biological action of OMP versus lisinopril was lower serum creatinine and blood urea nitrogen consistent with greater renoprotection. This renoprotective effect was attributed to the inhibition of renal NP degradation, which is known to enhance both GFR and sodium excretion as well as urinary cGMP, the second messenger of NPs. This notion is supported by the findings of Chen et al. (10) showing that inhibition of the NP receptor with HS-142 markedly attenuated the cardiorenal actions of OMP in dogs with experimental CHF.

When OMP was administered for longer periods into cardiomyopathic hamsters, it reduced cardiac hypertrophy and improved survival more than ACE or NEP inhibitors alone. Similarly, a 4-wk treatment with fasidrotiril to rats with myocardial infarction reduced cardiac hypertrophy significantly and more efficiently than captopril (5). Most recently, Bäcklund et al. (3) demonstrated that rats with myocardial infarction treated with OMP had less cardiomyocyte apoptosis and less adverse cardiac remodeling compared with rats treated with selective inhibitors of ACE or NEP. In clinical CHF, data indicate that OMP is either equipotent to classic ACE inhibitors or slightly superior to them. A 12-wk treatment with OMP improved the functional and hemodynamic status, including a reduction in afterload and central blood volume in patients with symptomatic heart failure, in a dose-dependent manner (18). When compared with lisinopril, it did not exhibit an advantage to the latter in the primary end point of exercise capacity but showed a positive trend in favor of the former in terms of the combined end point of death or hospital admission for worsening heart failure (27). Thus OMP exerted an improvement in patients classification according to NYHA criteria. Finally, of interest and of no less importance was our observation concerning the chronic effects of OMP and enalapril on cardiac hypertrophy in this experimental model of heart failure. The present study demonstrates that early or late treatment with these agents significantly attenuated the increase in cardiac mass in rats with ACF. A similar trend was reported by Marie et al. (17) in rats with CHF due to coronary artery ligation. In a similar model of CHF, Maki and co-workers (16) reported that treatment with sampatriat, another VPI, improved and actually prevented cardiac remodeling at least by direct inhibition of cardiac fibrosis. In previous studies, we demonstrated that the RAAS plays a major role in the pathogenesis of cardiac hypertrophy in this model of CHF (7). Besides exerting mechanical stress on the myocardium via increasing the afterload, angiotensin II plays a major role in myocardial remodeling of the failing heart (13, 36). The hypertrophic activity of RAAS is thought to involve both circulatory and local components of this system, which either directly or indirectly, by inducing growth factors in myocytes, increases cardiac fibroblast proliferation and fibrosis (13, 36). A decade ago, we demonstrated that rats with ACF are characterized by increased myocardial expression of renin and ACE mRNA in proportion to the severity of cardiac dysfunction (7, 25). Previously, we have demonstrated that inhibition of RAAS at the ACE level or angiotensin II has been found to be effective in reducing cardiac mass in experimental (7, 25) and clinical (22, 24, 26, 30) CHF. Early treatment with an angiotensin II antagonist has been shown to reduce cardiac hypertrophy and to inhibit collagen deposition in rats with myocardial infarction (30). Our findings that OMP was equipotent, or even slightly superior, to enalapril in attenuating the increase in cardiac mass support the concept that RAAS is a key player in cardiac remodeling and inhibition of this system by either ACE inhibitor or VPIs could serve as therapeutic tool for the treatment of CHF. Our findings that late treatment with either OMP or enalapril reduce established cardiac hypertrophy to the same extent as early treatment lend support to the assumption that both agents are useful for the regression of cardiac remodeling in early stages of CHF and in advanced stages of the disease.

Recently, the Omapatrilat Versus Enalapril Randomized Trial of Utility in Reducing Events study showed that OMP is favorable in heart failure but not more than ACE inhibitors (23). This finding, combined with the observation that OMP induced a higher rate of angioedema, dramatically reduced the initial enthusiasm from OMP. Our finding that OMP was specifically effective in decompensated CHF, characterized by severe renal dysfunction in maintaining sodium homeostasis, but not in compensated CHF, indicates that this drug should be specifically targeted to patients with heart failure with renal impairment in sodium balance.

In summary, the unique renoprotective action of OMP may have clinical implication to the treatment of CHF, particularly when associated with renal dysfunction.

REFERENCES

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