Antihypertensive and Antihypertrophic Effects of Omapatrilat in SHR

Louise M. Burrell, Joep Droogh, Olivier Man in’t Veld, Melinda D. Rockell, Nicole K. Farina, and Colin I. Johnston

Vasopeptidase inhibitors, such as omapatrilat are single molecules that simultaneously inhibit neutral endopeptidase (NEP) and angiotensin converting enzyme (ACE). In normotensive rats, a single dose of oral omapatrilat (10 mg/kg) and 1 mg/kg inhibited plasma ACE ($P < .01$) for 24 h and increased plasma renin activity for 8 h ($P < .01$). In vitro autoradiography using the specific NEP inhibitor radioligand $^{125}$I-RB104 and the specific ACE inhibitor radioligand $^{125}$I-MK351A showed omapatrilat (10 mg/kg) caused rapid and potent inhibition of renal NEP and ACE, respectively, for 24 h ($P < .01$).

In spontaneously hypertensive rats, 10 days of oral omapatrilat (40 mg/kg/day) reduced blood pressure (vehicle 237 ± 4 mm Hg; omapatrilat, 10 mg/kg, 212 ± 4 mm Hg; omapatrilat 40 mg/kg, 197 ± 4 mm Hg, $P < .01$) in a dose-dependent manner (10 v 40 mg/kg, $P < .01$). Left ventricular hypertrophy was significantly reduced by high-dose omapatrilat (vehicle 2.76 ± 0.03 mg/g body weight; omapatrilat, 10 mg/kg, 2.71 ± 0.02 mg/g; omapatrilat 40 mg/kg, 2.55 ± 0.02 mg/g, $P < .01$) and omapatrilat also increased kidney weight compared to vehicle (both doses, $P < .01$).

Omapatrilat caused significant inhibition of plasma ACE and increased plasma renin activity (both doses, $P < .01$), and in vitro autoradiographic studies indicated sustained inhibition of renal ACE and NEP (both doses, $P < .01$).

Omapatrilat is a potent vasopeptidase inhibitor, and its antihypertensive effects are associated with inhibition of NEP and ACE at the tissue level and beneficial effects on cardiovascular structure. Relating the degree of tissue inhibition to physiologic responses may allow further definition of the role of local renin angiotensin and natriuretic peptide systems in the beneficial effects of vasopeptidase inhibitors. Am J Hypertens 2000; 13:1110–1116 © 2000 by the American Journal of Hypertension, Ltd.

Vasopeptidase inhibitors are single molecules that simultaneously inhibit the enzyme neutral endopeptidase (NEP) and angiotensin converting enzyme (ACE), and represent a novel therapeutic approach for the treatment of cardiovascular disease.\textsuperscript{1,2} Inhibition of NEP, the major enzymatic pathway for degradation of natriuretic peptides, leads to an increase in the vasorelaxant, diuretic, and natriuretic effects of the natriuretic peptides,\textsuperscript{3} whereas inhibition of ACE prevents...

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the formation of the vasoconstrictor, antinatriuretic, and trophic hormone angiotensin II.

Simultaneous inhibition of NEP and ACE may thus offer potential advantages over inhibition of either enzyme alone and compounds that simultaneously inhibit both enzymes have now been rationally designed taking into account the similar structural characteristics of the catalytic site of both peptidases. Several such inhibitors are available including omapatrilat, which in vitro has similar potency to inhibit both rat renal NEP ($K_i = 9 \text{ nmol/L}$) and rabbit lung ACE ($K_i = 6 \text{ nmol/L}$).

In experimental hypertension, the blood pressure-lowering effects of vasopeptidase inhibitors are independent of volume or renin status and greater than that produced by the individual inhibitors alone. However, simply controlling blood pressure is no longer a sufficient goal in the management of hypertension. It is an advantage that any new antihypertensive agent not only reduce blood pressure but also prevent or regress end-organ damage such as left ventricular hypertrophy, vascular hypertrophy, and renal failure. In addition, it is likely that many of the beneficial effects of ACE inhibitors on target organ damage relate to inhibition of tissue ACE.

To date few studies have assessed the ability of vasopeptidase inhibitors to regress target organ damage or to inhibit ACE in organs of cardiovascular relevance such as the kidney and the heart. The effect of vasopeptidase inhibitors to inhibit tissue NEP is also of importance as the natriuretic peptide system is recognized not only as an endocrine but also a paracrine system. By relating the degree of tissue inhibition to physiologic effects, it may be possible to determine the role of local natriuretic peptide systems in the beneficial effects of NEP inhibitors.

In this study the potency and duration of action of omapatrilat, a potent balanced vasopeptidase inhibitor to block the circulating renin angiotensin system, and inhibit renal tissue NEP and ACE in normotensive rats was assessed. Next, the effect of omapatrilat on systolic blood pressure in spontaneously hypertensive rats (SHR) was assessed along with its effects on cardiac functional parameters, and inhibition of ACE and NEP in tissues of physiologic importance such as kidney and heart.

**METHODS**

Experimental procedures were performed according to the National Health and Medical Research Council of Australia guidelines for animal experimentation. Male Sprague Dawley rats and SHR (200 to 250 g) were obtained from the Austin and Repatriation Medical Centre animal house. To confirm the inbred status of SHR, all rats are regularly tested with polymorphic markers. Animals were housed at 23°C to 25°C in a 12-h light–dark cycle with access to a standard rat chow (0.6% sodium, 2% chloride, 2% calcium; Norco, Melbourne, Australia) and tap water ad libitum. Omapatrilat (BMS 186716) a vasopeptidase inhibitor ($K_i$ values, NEP, 9 nmol/L; ACE, 6 nmol/L) was a gift from Bristol-Myers Squibb Pharmaceuticals, Princeton, NJ.

**In Vitro Autoradiography** Frozen tissue sections (20 μm) were cut on a cryostat (Microm, Walldorf, Germany) at −20°C, thaw mounted onto 1% gelatin-coated slides, and dessicated at 4°C overnight to remove moisture before freezing at −80°C. The specific radioligand $^{125}$I-RB104 ($K_i = 30 \text{ pmol/L}$) was used for NEP and $^{125}$I-MK351A RB104 ($K_i = 30 \text{ pmol/L}$) for ACE autoradiography. Quantiﬁcation of binding density was determined by computerized densitometry using radioactive standards that were corrected for decay and fitted to calibration curves to convert the optical density of the autoradiographs to dpm per mm². Results are expressed as a percentage of binding in vehicle-treated rats.

**Biochemical Analysis** Plasma renin activity (PRA) was measured by radioimmunoassay. Plasma atrial natriuretic peptide (ANP) was measured after florisil extraction by radioimmunoassay, and plasma ACE was measured by a fluorometric assay. Plasma sodium and osmolality were measured using an ion-selective electrode (ILyte, Instrumentation Laboratory, Milan, Italy) and a Wescor 5100C Vapour Pressure Osmometer (Wescor Inc., Logan, Utah), respectively. Plasma aldosterone was measured by radioimmunoassay using a commercially available kit (Diagnostic Products Corporation, Los Angeles, CA).

**In Vivo Dose Response With Omapatrilat** To assess the degree of inhibition of tissue and circulating ACE and tissue NEP after a single oral dose of omapatrilat, Sprague Dawley rats were weighed and then gavaged with vehicle (5% arabic gum) or omapatrilat (0.1, 1, 10 mg/kg) (n = 6 rats/group). Rats were killed by decapitation at 1 h after gavage. Trunk blood was collected into prechilled tubes containing EDTA/aprotinin (kallikrein inhibitor 500 U/mL) for the measurement of PRA and into prechilled heparin tubes (containing 100 μL of sodium heparin, 25,000 U/L) for the measurement of plasma ACE. Kidneys were snap frozen at −40°C and used to assess tissue NEP and ACE inhibition using in vitro autoradiography.

**In Vivo Time Course With Omapatrilat** To determine the length of action of omapatrilat on tissue and circulating ACE and tissue NEP after a single oral dose, Sprague Dawley rats were weighed and gavaged with vehicle (5% arabic gum) or omapatrilat (1 mg/kg and 10 mg/kg) (n = 3 to 6 rats/group). Rats were killed by decapitation at 1, 2, 4, 8, 18, and 24 h.
after gavage. Trunk blood was collected for the measurement of plasma ACE, PRA, and ANP, and kidneys snap frozen for assessment of tissue NEP and ACE inhibition using in vitro autoradiography.

**Chronic Effects of Omapatrilat in SHR** The chronic effect of oral omapatrilat on hemodynamic, metabolic, hormonal, and structural parameters was evaluated in adult male SHR (age, 16 weeks). Baseline systolic blood pressure (SBP) was measured by tail-cuff plethysmography (38L flatbed recorder, model 229 Amplifier, IITC Life Science, Woodland Hills, Texas) in conscious, lightly restrained rats. Rats were randomized to receive either vehicle or omapatrilat (40 mg/kg) (n = 10/group) by gavage for 10 days. The doses were chosen on the basis that our initial studies (described previously) indicated suppression of NEP and ACE with omapatrilat 10 mg/kg, and an earlier report in SHR showed that the equivalent of 40 mg/kg (100 μmol/L) lowered blood pressure over a 12-day period.4 Body weight and SBP were measured 1 h after gavage on days 1, 3, 7, and 10. In the second week of treatment rats were placed in metabolic cages for 24 h to assess the effect of treatment on urinary volume and sodium. On day 10, rats were dosed and killed 1 h later by decapitation and trunk blood collected for the measurement of plasma ACE, PRA, ANP, aldosterone, and osmolality. Kidneys were snap frozen for the assessment of renal NEP and ACE inhibition using in vitro autoradiography. Whole heart was removed, weighed and dissected into the left ventricle, which was weighed and then snap frozen for assessment of cardiac ACE inhibition using in vitro autoradiography.

**Statistical Analysis** Results are presented as mean ± SEM and data has been analyzed using one-way analysis of variance and the Fisher’s test where appropriate. When the data consisted of repeated measures at successive time points ANOVA for repeated measures was used to identify significant between-group differences. Significant differences were obtained when P < .05.

**RESULTS**

**In Vivo Dose Response** The changes in plasma ACE and renin activity, and tissue enzyme inhibition after a single oral dose of omapatrilat are shown in Table 1. All doses of omapatrilat suppressed plasma ACE compared to vehicle (P < .01) with 10 mg/kg producing significantly greater inhibition compared to 0.1 mg/kg dose (P < .01; Table 1). In contrast, only the high dose of omapatrilat (10 mg/kg) increased plasma renin activity compared to vehicle (P < .01; Table 1). Omapatrilat (0.1 and 1 mg/kg) had no effect on renal NEP or ACE, but 10 mg/kg significantly inhibited both renal NEP and ACE compared to vehicle (P < .01; Table 1).

**In Vivo Time Course** The time course of changes in plasma ACE, PRA, and ANP after gavage with omapatrilat (1, 10 mg/kg) are shown in Fig. 1. Oral omapatrilat suppressed plasma ACE for 24 h (P < .01), increased PRA for 8 h (P < .01), but neither dose altered plasma ANP. At the tissue level, high dose of omapatrilat (10 mg/kg) inhibited renal NEP and ACE for 24 h compared to vehicle (P < .01; Fig. 2).

**Chronic Effects of Omapatrilat in SHR** Baseline SBP was similar in all groups (vehicle 224 ± 1 mm Hg; omapatrilat (10 mg/kg) 223 ± 3 mm Hg; omapatrilat (40 mg/kg) 222 ± 2 mm Hg), and changed significantly with time and treatment (Fig. 3). On day 1 of treatment, SBP in the high-dose omapatrilat (40 mg/kg) group was 214 ± 5 mm Hg, which was significantly lower than vehicle (232 ± 3 mm Hg) and low-dose omapatrilat (10 mg/kg, 232 ± 3 mm Hg) (P < .01). On day 10 of treatment, both doses of omapatrilat (10, 40 mg/kg/day) reduced blood pressure compared to vehicle (vehicle 237 ± 4 mm Hg; omapatrilat, 10 mg/kg, 212 ± 4 mm Hg; omapatrilat 40 mg/kg, 197 ± 4 mm Hg, P < .01) in a dose-dependent manner (10 v 40 mg/kg, P < .01; Fig. 3).

### Table 1. Effect of a Single Oral Dose of Omapatrilat on Circulating and Tissue ACE and Tissue NEP in Normotensive Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vehicle</th>
<th>0.1 mg/kg</th>
<th>1 mg/kg</th>
<th>10 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma ACE (nmol/L/mL/min)</td>
<td>75 ± 5*</td>
<td>24 ± 5*</td>
<td>14 ± 3*</td>
<td>11 ± 1*</td>
</tr>
<tr>
<td>Plasma renin activity (nmol Angl/L/h)</td>
<td>3 ± 1</td>
<td>5 ± 1</td>
<td>13 ± 4</td>
<td>93 ± 15*</td>
</tr>
<tr>
<td>Renal ACE (% of vehicle)</td>
<td>100 ± 6</td>
<td>128 ± 11</td>
<td>119 ± 10</td>
<td>31 ± 13*</td>
</tr>
<tr>
<td>Renal NEP (% of vehicle)</td>
<td>100 ± 6</td>
<td>90 ± 12</td>
<td>83 ± 15</td>
<td>19 ± 7*</td>
</tr>
</tbody>
</table>

ACE = angiotensin converting enzyme; NEP = neutral endopeptidase.

Values are given as the mean ± SEM (n = 6/group).

* P < .01 v vehicle.
High-dose omapatrilat (40 mg/kg) caused a decrease in total heart weight (Table 2) and left ventricular mass compared to vehicle and low-dose omapatrilat ($P < .01$; Fig. 4, Table 2). Both doses of omapatrilat increased renal mass compared to vehicle ($P < .01$; Fig. 4, Table 2).

The effects of omapatrilat on metabolic parameters and plasma hormones are summarized in Table 2. Omapatrilat (40 mg/kg) increased urine volume compared to vehicle ($P < .01$) and there was a trend for urinary sodium to increase, which did not reach statistical significance. Omapatrilat had no effect on plasma osmolality but dose-dependently inhibited plasma ACE ($P < .01$) and increased PRA ($P < .01$). Plasma ANP and aldosterone were unchanged with omapatrilat (Table 2), which significantly inhibited renal NEP and ACE in a dose-dependent manner compared to vehicle ($P < .01$; Table 3). In addition, both doses of omapatrilat inhibited cardiac ACE compared to vehicle ($P < .01$).

FIG. 1. Time course effects of a single oral dose of omapatrilat (1, 10 mg/kg) on plasma (a) ACE, (b) renin activity and (c) ANP in normotensive Sprague Dawley rats. Bars indicate mean ± SEM, $n = 6$-6/group. **$P < .01$ v corresponding control (at time 0). ACE = angiotensin converting enzyme; ANP = atrial natriuretic peptide.

FIG. 2. Time course effects of a single oral dose of omapatrilat (1, 10 mg/kg) on renal (a) NEP and (b) ACE in normotensive Sprague Dawley rats. Bars indicate mean ± SEM, $n = 3-6$ group. **$P < .01$ v corresponding control (at time 0). NEP = neutral endopeptidase; ACE = angiotensin converting enzyme.

FIG. 3. Effect of chronic oral doses of omapatrilat on systolic blood pressure in the spontaneously hypertensive rats. Symbols indicate mean ± SEM, $n = 10$/group. **$P < .01$ v vehicle; §§$P < .01$ v omapatrilat (10 mg/kg).
DISCUSSION

The results of this study indicate that the vasopeptidase inhibitor omapatrilat inhibits renal NEP and ACE after oral dosing in the normotensive rat in a dose- and time-dependent manner. In SHR, omapatrilat reduces blood pressure, regresses left ventricular hypertrophy, and inhibits renal NEP, and renal and cardiac ACE.

Many studies of vasopeptidase inhibitors have used relatively indirect measures to assess the degree of NEP inhibition (ie, increased urinary/plasma cGMP or ANP). The current study used quantitative in vitro autoradiography and a selective radiolabeled inhibitor of NEP to localize NEP in kidney and to quantitate the degree of inhibition after administration of omapatrilat. It was clearly shown that omapatrilat caused rapid and prolonged inhibition of renal NEP at the site of highest abundance of NEP, namely the proximal tubules of the kidney; renal NEP was inhibited by 95% within 1 h of oral dosing, and such effects persisted for 24 h. With chronic dosing in the SHR, a dose-dependent effect on renal NEP was seen with 69% inhibition with low-dose (10 mg/kg) and 100% inhibition of renal NEP with high-dose (40 mg/kg) omapatrilat.

In the time course and dose response study, omapatrilat (1 and 10 mg/kg) caused rapid and prolonged suppression of circulating ACE, although only 10 mg/kg omapatrilat caused renal ACE inhibition, suggesting that inhibition of the circulating renin angiotensin system (RAS) does not necessarily translate to inhibition at the tissue level. As the long-term benefits of the ACE inhibitors in preventing or reversing target organ damage in hypertension depends on their ability to inhibit the tissue RAS, it is important in experimental models of disease to confirm the potency of compounds to directly inhibit tissue ACE activity.

In the 10-day dosing study in SHR, omapatrilat had a rapid onset of action to reduce blood pressure with high-dose omapatrilat causing a 1.6-fold greater decrease in pressure compared to low dose as well as causing a significant diuresis. Although low-dose omapatrilat (10 mg/kg) significantly inhibited both renal NEP (69%) and ACE (87%), high-dose omapatrilat (40 mg/kg) caused complete inhibition of both enzymes. Given the biologic actions of the natriuretic peptides, one can speculate that inhibition of tissue NEP and consequent changes in tissue levels of the natriuretic peptides played a role in the greater renal effects as well as the improvement in blood pressure observed with high- compared to low-dose omapatrilat.

The benefits of omapatrilat to regress cardiac hypertrophy confirms other reports using the vasopeptidase inhibitors S21402 and CGS 30440, which also regress cardiac hypertrophy in association with sustained reductions in blood pressure. However, this study is the first to demonstrate the rapid (within 10 days) effect of blood pressure lowering with a vasopeptidase inhibitor on cardiac hypertrophy. It is not clear from this study what the relative contribution of cardiac ACE versus NEP inhibition has to play in the antihypertrophic effects of omapatrilat. Cardiac ACE was reduced to a similar degree by both doses of omapatrilat, but because of technical difficulties it was not possible to measure the degree of cardiac NEP inhibition in this study. Although the reduction in cardiac hypertrophy is in part mediated by a decrease

<table>
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<tr>
<th>TABLE 2. EFFECT OF ORAL OMAPATRILAT ON ORGAN WEIGHTS, METABOLIC PARAMETERS, AND PLASMA HORMONES IN THE SHR</th>
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</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
<td>Organ weight</td>
</tr>
<tr>
<td>Heart mass (mg/g)</td>
</tr>
<tr>
<td>Left ventricular mass (mg/g)</td>
</tr>
<tr>
<td>Renal mass (mg/g)</td>
</tr>
<tr>
<td>Metabolic effects</td>
</tr>
<tr>
<td>Urine volume (mL/100 g)</td>
</tr>
<tr>
<td>Urinary sodium (µmol/min/100 g)</td>
</tr>
<tr>
<td>Biochemical/hormonal data</td>
</tr>
<tr>
<td>Plasma osmolality (mOsm/kg)</td>
</tr>
<tr>
<td>Plasma ACE (nmolHL/mL/min)</td>
</tr>
<tr>
<td>Plasma renin activity (nmol AngI/mL/h)</td>
</tr>
<tr>
<td>Plasma ANP (pmol/L)</td>
</tr>
<tr>
<td>Plasma aldosterone (pmol/L)</td>
</tr>
</tbody>
</table>

SHR = spontaneously hypertensive rats; ANP = atrial natriuretic peptide; other abbreviation as in Table 1.
Values are given as the mean ± SEM (n = 10/group).
* P < .01 v vehicle; † P < .05; ‡ P < .01 v omapatrilat (10 mg/kg).
in cardiac afterload, there is also evidence to suggest that nonhemodynamic factors may influence myocardial growth and extracellular matrix deposition independent of blood pressure changes.16–18 In the present study, omapatrilat (40 mg/kg) caused a 4.2-fold greater decrease in cardiac hypertrophy than the low dose compared with only a 1.6-fold difference in blood pressure between the two doses. This result lends further support to the idea that nonhemodynamic factors also influence cardiac growth. As the natriuretic peptides act to antagonize the growth-promoting actions of angiotensin II, augmentation of their actions through NEP inhibition may have beneficial effects on the heart independent of blood pressure changes. Certain 4 weeks of treatment with the NEP inhibitor SCH34826 reduced left ventricular hypertrophy in the absence of blood pressure changes19 and there is also evidence to suggest that ANP has antiproliferative and apoptotic properties in cardiac myocytes.20,21

Both NEP and ACE play a role in the inactivation of kinins in the rat,22 suggesting that the cardioprotective effects of vasopeptidase inhibitors may be mediated in part through increased bradykinin levels. Certainly when membranes from infarcted rat hearts were incubated with synthetic bradykinin, omapatrilat increased the half-life of bradykinin over and above that observed with enalaprilat,23 and preliminary data in humans suggests that omapatrilat is superior to ACE inhibition in improving both symptoms and mortality/morbidity in heart failure.24

To summarize, vasopeptidase inhibitors lower blood pressure irrespective of the renin or volume status, and therefore, should be effective as first-line agents in a wide range of patients. The current study showed that the vasopeptidase inhibitor omapatrilat causes a rapid and prolonged suppression of the circulating RAS and renal NEP and ACE, and its antihypertensive effects in the SHR are maintained long term and are associated with beneficial effects on cardiac hypertrophy. If the benefits of NEP inhibition extend beyond potentiation of the efficacy of ACE inhibition to lower blood pressure and include antiproliferative and antitrophic effects, vasopeptidase inhibitors such as omapatrilat are likely to be a powerful addition to our current therapeutic approaches in the management of cardiovascular disease.

**REFERENCES**


**TABLE 3. EFFECT OF ORAL OMAPATRILAT ON TISSUE ENZYME INHIBITION IN THE SHR**

<table>
<thead>
<tr>
<th>Tissue Enzyme (% of vehicle)</th>
<th>Vehicle</th>
<th>Omapatrilat (10 mg/kg)</th>
<th>Omapatrilat (40 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal NEP</td>
<td>100 ± 5</td>
<td>31 ± 9*</td>
<td>0†§</td>
</tr>
<tr>
<td>Renal ACE</td>
<td>100 ± 4</td>
<td>13 ± 5*</td>
<td>0†§</td>
</tr>
<tr>
<td>Left ventricular ACE</td>
<td>100 ± 6</td>
<td>15 ± 1*</td>
<td>14 ± 2*</td>
</tr>
</tbody>
</table>

Abbreviations as in Tables 1 and 2.
Values are given as the mean ± SEM (n = 4/group).
* P < .01 v vehicle; † P < .05; ‡ P < .01 v omapatrilat (10 mg/kg).


