Physiologic Consequences of Vasopeptidase Inhibition in Humans: Effect of Sodium Intake

MICHEL AZIZI, MAXIME LAMARRE-CLICHE, AGNÈS LABATIDE-ALANORE, ALVINE BISSERY, THAN TAM GUYENE, and JOËL MÉNARD
Clinical Investigation Center 9201, Assistance Publique des Hôpitaux de Paris/INSERM, Hôpital Européen Georges Pompidou, Paris, France.

Abstract. The in vivo inhibition of angiotensin-converting enzyme (ACE) and neutral endopeptidase (NEP) were monitored simultaneously by sequentially measuring the urinary excretion of N-Acetyl-Ser-Asp-Lys-Pro and of the atrial natriuretic factor to compare the magnitude and the duration of action of a vasopeptidase inhibitor, omapatrilat, and an ACE inhibitor, fosinopril. Single oral doses of 40 or 80 mg of omapatrilat or 20 mg of fosinopril were administered to 24 normotensive, sodium-depleted or -replete volunteers in a placebo-controlled crossover study. ACE inhibition persisted longer after treatment with omapatrilat than with fosinopril, and there was no major difference between the effects of 40 and 80 mg of omapatrilat. The duration of NEP inhibition by omapatrilat was shorter than that of ACE inhibition. Although omapatrilat effectively inhibited NEP, it had a mild and transient natriuretic effect and did not increase natriuresis more than fosinopril. Omapatrilat induced a decrease in BP and an increase in plasma renin more rapidly and more effectively than fosinopril. The BP and renin effects of omapatrilat persisted despite high sodium intake, which neutralized the effects of fosinopril. The simultaneous inhibition of ACE and NEP may be more effective in reducing BP than the inhibition of ACE alone and less dependent on sodium balance.

Omapatrilat is a vasopeptidase inhibitor that has a similar nanomolar inhibitory constant for both neutral endopeptidase (NEP) and angiotensin I-converting enzyme (ACE) in vitro (1). This drug is designed to treat hypertension (2) and congestive heart failure (3,4). In animal models, NEP inhibition may have additional beneficial effects on target organs damage beyond ACE inhibition (5–8). Omapatrilat was shown to have a greater antihypertensive efficacy in hypertensive patients than ACE inhibitors, such as lisinopril (9,10) or enalapril (11). Vasopeptidase inhibition could have an advantage over selective ACE inhibition for the treatment of patients with congestive heart failure. However, the beneficial effect of omapatrilat on cardiovascular morbidity compared with lisinopril in patients with congestive heart failure suggested by the IMPRESS study (4) has not been confirmed by the results of the OVERTURE study (12), in which no significant difference in terms of cardiovascular morbidity or mortality between 40 mg of omapatrilat daily and 10 mg of enalapril twice daily was reported.

We have previously reported the BP and hormonal effects of a single oral dose of 10 mg of omapatrilat in sodium-depleted normotensive subjects (13). Omapatrilat (10 mg) had a shorter BP-lowering effect than a single oral dose of 20 mg of fosinopril, despite the fact that, at the dose used, both drugs appeared to have a similar potency in inhibiting ACE, whereas omapatrilat, by inhibiting NEP, increased urinary atrial natriuretic peptide (ANP) and blunted the decrease in plasma ANP due to ACE inhibition (13).

The doses of omapatrilat subsequently used in the clinical development program were higher (up to 80 mg), and how an increase in dose modifies the hormonal status and hemodynamic effects of the drug by comparison with ACE inhibition remains unknown.

To investigate the differences between vasopeptidase and ACE inhibition, we compared the effects of single oral doses of 40 and 80 mg of omapatrilat with the effects of the usual daily dose of 20 mg of fosinopril in sodium-replete and -depleted normotensive subjects. The use of a high sodium status reduces the importance of the renin-angiotensin system (RAS) in BP control while possibly increasing the vascular and renal homeostatic role of ANP, whereas the reverse should be obtained in subjects with a low sodium status. Moreover, the use of 40-mg and 80-mg doses of omapatrilat may moderately increase the magnitude and/or the duration of ACE inhibition, which already seemed to be close to the maximum level at the 10-mg dose (13) but which might greatly enhance the magnitude and/or the duration of NEP inhibition.

Materials and Methods

Study Design

A two-panel (high or low sodium), double-blind, placebo-controlled, randomized, four-period, crossover study design was used. Twenty-four healthy normotensive male volunteers (age, 18 to 35 yr) were assigned to the low- (n = 12) or high-sodium panel (n = 12) and treatments were assigned according to a Latin square design. After

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Correspondence to Dr. Michel Azizi, Centre d’Investigations Cliniques, Hôpital Européen Georges Pompidou, 20-40 rue Leblanc, 75908 Paris cedex 15, France. Phone: 33-1-56-09-29-11; Fax: 33-1-56-09-29-29; Email: michel.azizi@egp.ap-hop-paris.fr
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giving written and informed consent, each subject received a single oral dose of 40 mg of omapatrilat (O40), 80 mg of omapatrilat (O80), 20 mg of fosinopril (F20), or matched placebos (P). Volunteers were hospitalized for 36 h when they received the study drugs or matched placebos on four occasions separated by 2-wk washout intervals. The protocol was approved by the “Comité Consultatif de Protection des Personnes se prêtant à des Recherches Biomédicales” (Paris-Cochin, France).

**Study Protocol**

The 12 subjects assigned to the low-sodium panel group were instructed to arrive for each period at the Clinical Investigation Center at 7 p.m. on the evening before the drugs or placebo were given (day 0). To induce mild sodium depletion, subjects were given 40 mg of furosemide at 9 p.m. on day 0 and received a sodium-restricted diet (30 mmol/d) through the 36 h of each period of hospitalization as described previously (13,14).

A second group of 12 normotensive volunteers entered the high-sodium panel (NaCl >150 mmol/d). They received slow-release sodium tablets (6 x 1 g) for 6 d (from day –5 to 0) and were instructed to select high-sodium foods preferentially. On the evening before the study (day 0), all of the subjects attended the Clinical Investigation Center at 7 p.m and continued their high-sodium diet through the 36 h of each period of hospitalization.

At 9 a.m. on the study day (day 1), subjects in both the low- and high-sodium panel received a single oral dose of O40, O80, F20, or placebo with 150 ml of water according to the randomization schedule after 1 h of rest in a semirecumbent position to allow for equilibration of BP, heart rate, and hormones. To promote urine production, subjects were given an additional 100 ml of water immediately after receiving the dose and 250 ml 1 h later. Fluid intake was unrestricted on the study days (1500 to 2000 ml/24 h). Subjects remained in a resting, semirecumbent position for blood samplings and BP measurements. Blood was sampled before dose and 1, 2, 4, 6, 12, and 24 h after dose for plasma active renin, ACE activity, ANP, endothelin 1 (ET1), and Big-ET1 determinations. Mean arterial pressure (MAP) (average of 10 measurements performed at 2-min intervals) was determined before dose and 1, 2, 4, 6, 12, and 24 h after dose with an automatic BP recorder (Press Mate BP 8800; Colin Co., Komaki-City, Japan). Subjects completed two 6-h urine collections (from 9 p.m. on day 0 to 9 a.m. on day 1) before drug intake and five urine collections afterwards (three 4-h collections starting at 9 a.m. on day 1 and two 6-h urine collections starting at 9 p.m. on day 1). Volume, electrolytes, ANP, and N-Acetyl-Ser-Asp-Lys-Pro (AcSDKP) were measured in each urine sample.

**Laboratory Methods**

The methods used to determine ex vivo plasma ACE activity (using the synthetic substrate Hip-His-Leu in the Cushman assay), plasma active renin and plasma and urine ANP levels and those used for blood and urine samplings were as described previously (13).

Urine ANP and AcSDKP were sequentially measured to monitor simultaneously the kinetics of the in vivo inhibition of NEP and ACE. Urine AcSDKP concentration was determined by a competitive enzyme immunoassay (15) and was used as a marker of in vivo tubular ACE inhibition (16).

Plasma ET1 and Big-ET1 concentrations were measured by an enzyme immunoassay (kit QET00; QuantiGlo, Abingdon, UK; kit BI-20072; Bioadvance, Emerainville, France).

**Statistical Analyses**

Data were analyzed using crossover design ANOVA models to test for the effects of the treatment, period, sequence, and carryover. When the F-test was significant (P < 0.05) and when there was no period sequence or carryover effect, paired comparisons were made between specific treatments using the Newman-Keuls test.

Stata Statistical Software (Release 7.0; Stata Corp., College Station, TX) was used for statistical analyses. Data are expressed as mean ± SD, unless otherwise specified. P < 0.05 is considered to be significant.

**Results**

No period, carryover, or sequence effects were detected for any of the results. Therefore, only treatment effects are reported in the text and tables.

**Time Course of ACE and NEP Inhibition**

**Ex Vivo Plasma ACE Activity.** The effects of omapatrilat and fosinopril on ex vivo plasma ACE activity were independent of sodium intake. At their peaks, both omapatrilat and fosinopril induced almost complete inhibition of ex vivo ACE activity (O80, 100%; O40, 100%; F20, 99 ± 2%). At 24 h after dose, ACE was more inhibited by O80 (88 ± 5%) or O40 (87 ± 3%) than by F20 (43 ± 12%; P < 0.05; Figure 1). In 26 cases (both panels), plasma ACE activity could be measured 48 h after drug intake and confirmed the longer duration of inhibition after O80 (82 ± 6%; n = 10) and O40 (76 ± 7%; n = 6) than after F20 (23 ± 15%; n = 10). Moreover, there was no significant difference in ex vivo plasma ACE activity between the two doses of omapatrilat either at peak or up to 48 h after treatment.

**Sequential Changes in Urine AcSDKP and ANP Excretion.** Basal AcSDKP excretion was low and not influenced by sodium status (low salt, 0.94 ± 0.67 nmol/h; high salt, 0.96 ± 0.54 nmol/h). Independently of the sodium status, O80, O40, and F20 massively and rapidly increased urine AcSDKP excretion within the first 4 h of drug intake. Urine AcSDKP excretion then slightly decreased from the 8th to 12th hours onward and remained much higher than baseline and placebo concentrations even in the last urine sample collected (18 to 24 h after treatment; Figure 2). In both the low- and the high-salt panels.

**Figure 1.** Time course of ex vivo plasma angiotensin-converting enzyme (ACE) activity in normotensive subjects in the low- and high-salt panels. —, placebo; □, 80 mg of omapatrilat; ▲, 40 mg of omapatrilat; ○, 20 mg of fosinopril.
high-salt panel groups, there was no difference between O40 and O80 in terms of cumulative urine AcSDKP excretion. Both doses increased significantly more urine AcSDKP excretion than F20 (Figure 2).

Urine ANP Excretion. As expected, baseline urine ANP excretion was significantly higher in the high-sodium panel group (12 ± 4 ng/12 h) than in the low-sodium panel group (6 ± 2 ng/12 h; P < 0.001) (Tables 1 and 2).

In both the high- and low-sodium panel groups, O80 and O40 massively and significantly increased urine ANP excretion when compared with F20 and placebo, whereas F20 did not change urine excretion of ANP when compared with placebo (Figure 2). Urine ANP excretion rapidly increased within the first 4 h after omapatrilat intake and then decreased from the 4th hour onward to reach the same level as the placebo in the last urine collection period (18 to 24 h; P = NS in both groups). The difference in 24-h cumulative urine ANP excretion between the two doses of omapatrilat was more marked in the high-sodium panel group (Figure 2).

Time Course of BP and Urine Sodium Excretion

Decrease in Mean Arterial Pressure. In the low-salt panel group, O80, O40, and F20 significantly decreased MAP from baseline compared with the placebo. The two doses of omapatrilat had the same effect, but the decrease in MAP after omapatrilat was significantly larger than after fosinopril intake, especially at peak (Table 3 and Figure 3).

The results for the high-sodium panel group were completely different. MAP fell slightly with F20 but did not reach statistical significance compared with placebo. The two doses of omapatrilat significantly decreased MAP at peak compared with both the placebo and F20 and at 24 h compared with placebo (Table 3 and Figure 3).

Urine Sodium Excretion and Urine Volume. In the low-salt panel group, O80 and F20, but not O40, had a slight transient and significant natriuretic effect compared with the placebo during the first 12 h after drug intake (Table 1 and Figure 4). The total amount of sodium excreted in the 24 h after drug intake was slightly but not significantly increased by O80 (73 ± 41 mmol), O40 (64 ± 34 mmol), and F20 (73 ± 37 mmol) compared with placebo (50 ± 36 mmol; F3,27 = 2.41; P = 0.09; Table 1). The natriuretic effect of O80 was of similar magnitude to that of F20.

In the high-salt panel group, the increase in natriuresis observed between the first (0 to 4 h) and third (8 to 12 h) collection periods after placebo administration was probably a consequence of the resting semirecumbent position adopted during the investigation periods (Figure 4). In this group, the total amount of sodium excreted in 24 h did not significantly differ between drugs and placebo and the slight and very transient natriuretic effect of O80 and F20 compared with the placebo during the first 4 h after drug intake did not achieve statistical significance in the ANOVA (F3,27 = 2.10; P = 0.12; Table 2).

None of the active drugs had a diuretic effect (data not shown).

Time Course of Hormonal Changes

Plasma Active Renin Changes. As expected, baseline plasma active renin concentrations were much higher in the low-salt group than in the high-salt group, averaging 38 pg/ml (range, 8 to 11 pg/ml) and 9 pg/ml (range, 34 to 42 pg/ml), respectively (Table 3 and Figure 3).

In the low-salt panel group, both active drugs massively increased plasma active renin levels from baseline, whereas they remained stable throughout the placebo period (Figure 3). The rise in plasma active renin concentration was significantly lower after treatment with F20 than after treatment with O40 or O80. As expected from the time course of ACE inhibition, the plasma renin concentration peaked later with fosinopril than with omapatrilat. The two doses of omapatrilat had the same effects on renin release through 24 h (Table 3).

In the high-salt panel group, the effects of both omapatrilat and fosinopril on renin release were blunted, but the trends were similar to those observed in the low-sodium panel group. The peak plasma active renin concentrations achieved with O80 were similar to those achieved with O40 and higher than those achieved with F20. All of the values were significantly higher than those achieved with the placebo (Table 3 and Figure 3).

Plasma ANP. As expected, baseline plasma ANP concentrations were significantly higher in the high-sodium group (57 ± 20 pg/ml) than in the low-sodium group (41 ± 13 pg/ml; P = 0.025), although the difference was less than that observed for plasma active renin concentrations.

The effect of omapatrilat on plasma ANP concentrations was

Figure 2. (Top) Time course of urine n-Acetyl-Ser-Asp-Lys-Pro (AcSDKP) excretion in normotensive subjects in the low- and high-salt panels. The low-salt panel group excreted 547 ± 117 nmol of AcSDKP in 24 h after O80, 495 ± 145 nmol after O40, 338 ± 105 nmol after F20, and 22 ± 10 nmol after receiving the placebo (P < 0.05). The high-salt panel group excreted 655 ± 159 nmol of AcSDKP in 24 h after receiving O80, 584 ± 119 nmol after O40, 441 ± 148 nmol after F20, and 33 ± 15 nmol after receiving the placebo (P < 0.05). (Bottom) Time course of urine atrial natriuretic peptide (ANP) excretion in normotensive subjects in the low- and high-salt panels. □, placebo; ■, 80 mg of omapatrilat; □, 40 mg of omapatrilat; □, 20 mg of fosinopril.
Table 1. Effects of a single oral dose of 80 mg of omapatrilat (O80), 40 mg of omapatrilat (O40), 20 mg of fosinopril (F20), and placebo (P) in normotensive subjects on the ANP and sodium urine excretion in the low-salt panel

<table>
<thead>
<tr>
<th>Time Interval (h)</th>
<th>Cumulative Excretion 0 to 24</th>
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<tbody>
<tr>
<td></td>
<td>ng/h</td>
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<tr>
<td>Low-Salt Panel</td>
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<tr>
<td>O80</td>
<td>0.46 ± 0.18</td>
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<td>O40</td>
<td>0.71 ± 0.37</td>
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<td>F20</td>
<td>0.9 ± 0.71</td>
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<td>P</td>
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<td>F-test (3;27)</td>
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<td>Urine sodium excretion</td>
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<td>O80</td>
<td>24 ± 6</td>
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<td>O40</td>
<td>26 ± 6</td>
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<td>F20</td>
<td>24 ± 9</td>
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<td>P</td>
<td>26 ± 8</td>
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<tr>
<td>F-test (3;27)</td>
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<tr>
<td>Urine sodium (change from baseline)</td>
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<tr>
<td>O80</td>
<td>0.92 ± 2.75</td>
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<tr>
<td>O40</td>
<td>0.11 ± 1.77</td>
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<tr>
<td>F20</td>
<td>1.64 ± 2</td>
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<tr>
<td>P</td>
<td>−0.5 ± 1.6d</td>
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<tr>
<td>F-test (3;27)</td>
<td>3.75a</td>
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</table>

F (3;27): a P < 0.05, b P < 0.01, c P < 0.001 by ANOVA, d P < 0.05 versus O80, e P < 0.05 versus O40, f P < 0.05 versus F20.
Effects of a single oral dose of O80, O40, F20, and P in normotensive subjects on the ANP and sodium urine excretion in the high-salt panel

### Table 2

<table>
<thead>
<tr>
<th>Time Interval (h)</th>
<th>Cumulative Excretion (ng/h)</th>
<th>ANP</th>
<th>Sodium</th>
<th>ANP</th>
<th>Sodium</th>
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</thead>
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### Discussion

In this study, we looked at a new method to monitor *in vivo* ACE and NEP inhibition simultaneously by measuring urinary AcSDKP (16) and ANP (17). We showed that omapatrilat appears to be a potent and long-acting ACE inhibitor and a shorter-acting NEP inhibitor. We showed that omapatrilat has different clinical effects on BP in normotensive subjects than the reference ACE inhibitor, fosinopril, at its usual daily dose. A high-sodium diet neutralizes the BP-lowering effect of the ACE inhibitor, but it does not suppress the effects of the two doses of the vasopetidase inhibitor, which were shown to be equipotent. There was no dose-response relationship for omapatrilat on the decrease in MAP, the renin response, or the *in vivo* plasma ACE inhibition, indicating that 40 mg of omapatrilat exerts the maximum pharmacodynamic effect on these parameters. However, NEP inhibition was slightly more sustained with 80 mg of omapatrilat than with 40 mg, as indicated by the fact that significantly more ANP was excreted in the urine with the high-dose than with the low-dose of omapatrilat. Despite a marked tubular NEP inhibition, neither of the doses of omapatrilat induced an additional natriuretic effect compared with 20 mg of fosinopril, which had no effect on urine ANP excretion. This suggests that the supplementary BP-lowering effect of NEP inhibition in addition to ACE inhibition is not mediated by a natriuretic effect after the ingestion of a single dose of the active compounds.

### Effects of Omapatrilat on ACE and NEP Inhibition

Although the maximal level of ACE inhibition was the same for the two doses of omapatrilat and for 20 mg of fosinopril, the duration of ACE inhibition by omapatrilat was significantly longer than that by fosinopril and largely exceeded 24 h. We have selected the usual daily dose of 20 mg of fosinopril to be consistent with our previous study that compared this dose to 10 mg of omapatrilat (13). Although, it is likely that a 40-mg dose of fosinopril would inhibit ACE for a longer period of
time, we know that in patients with mild to moderate hypertension no clear dose-response reduction in trough BP has been reported from 10 mg to 40 mg of fosinopril once daily (18) and that a 40-mg dose of fosinopril has only been shown to be slightly more effective in reducing the symptoms of dyspnea and the diuretic use than the 20-mg dose in patients with congestive heart failure (19).

The very prolonged duration of action of omapatrilat on plasma ACE and probably endothelial ACE is probably due to the very tight binding of omapatrilat to ACE active sites, especially to its C-terminal active site (20). It may also be explained in part by the pharmacokinetics of omapatrilat and its metabolites. Plasma radioactivity after dosing with radiolabeled omapatrilat declines very slowly in healthy subject, with a terminal half-life of about 8 to 9 d and a large steady-state volume of distribution (21 l/kg) suggestive of a deep pharmacokinetic compartment (21). In addition, one of the metabolites of omapatrilat, the L-cysteine mixed disulfide adduct, has a potential to revert to omapatrilat \textit{in vivo} and therefore prolong its biologic activity (22).

Contrasting with its prolonged inhibitory effects on ACE, omapatrilat inhibited NEP for less time than ACE. Urine ANP

\begin{table}[h]
\centering
\caption{Effects of a single oral dose of O8, O40, F20, and P in normotensive subjects in both the low-salt and the high-salt panel on plasma active renin and fall in mean arterial pressure (MAP).}
\begin{tabular}{|l|c|c|c|c|}
\hline
 & Baseline Value at Peak & Value 24 h after Dosing & AUC$_{0-24}$ \\
 & pg/ml & pg/ml & pg/ml & pg · h/ml \\
\hline
\hline
\textbf{Low-salt panel} & & & & \\
plasma active renin & pg/ml & pg/ml & pg/ml & pg · h/ml \\
O40 & 42 [34 to 51] & 370 [311 to 442] & 121 [93 to 157] & 4680 [3833 to 5714] \\
F20 & 35 [30 to 41] & 144 [112 to 184] & 68 [57 to 80] & 2028 [1626 to 2528] \\
F-test (3;27) & 0.98 ns & 74c & 174c & \\
fall in MAP & mmHg & mmHg & mmHg · h & \\
O80 & −15 ± 6 & −6 ± 5 & 186 ± 108 & \\
O40 & −17 ± 6 & −7 ± 5 & 219 ± 103 & \\
F20 & −13 ± 5 & −4 ± 3 & 160 ± 65 & \\
P & −6 ± 3 & −3 ± 4 & 60 ± 37 & \\
F-test (3;27) & 15c & 1.27 ns & 8c & \\
\hline
\textbf{High-salt panel} & & & & \\
plasma active renin & pg/ml & pg/ml & pg/ml & pg · h/ml \\
F-test (3;27) & 0.86 ns & 13c & 12c & 17c \\
fall in MAP & mmHg & mmHg & mmHg · h & \\
O80 & −12 ± 5 & −6 ± 4 & 150 ± 78 & \\
O40 & −13 ± 6 & −6 ± 6 & 154 ± 95 & \\
F20 & −8 ± 3 & −3 ± 4 & 89 ± 84 & \\
P & −4 ± 4 & −2 ± 4 & 46 ± 72 & \\
F-test (3;27) & 11c & 5b & 11c & \\
\hline
\multicolumn{4}{l}{\textsuperscript{a} Data expressed as geometric mean [95% CI]. AUC$_{0-24}$, area under curve from 0 to 24 h calculated according to the trapezoidal rule. F (3;27): \textsuperscript{b} P < 0.01, \textsuperscript{c} P < 0.001 by ANOVA. \textsuperscript{d} P < 0.05 versus O80; \textsuperscript{e} P < 0.05 versus O40; \textsuperscript{f} P < 0.05 versus F20.}
\end{tabular}
\end{table}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{chart.png}
\caption{(Top) Time course of the decrease in mean arterial pressure (MAP) in normotensive subjects in the low- and high-salt panels. (Bottom) Time course of plasma active renin concentration in normotensive subjects in the low- and high-salt panels. —, placebo; ■, 80 mg of omapatrilat; ▲, 40 mg of omapatrilat; ○, 20 mg of fosinopril.}
\end{figure}
concentration, a marker of tubular NEP inhibition, increased significantly 4 h after dose but returned to baseline levels within 12 to 18 h, even with the highest dose of omapatrilat (80 mg). This clearly contrasts with the long-lasting effect of omapatrilat on urine AcSDKP, a marker of tubular ACE inhibition measured in parallel with urine ANP. The use of these indirect markers of in vivo NEP and ACE inhibition revealed that the inhibition measured in parallel with urine ANP. The use of these indirect markers of in vivo NEP and ACE inhibition revealed that the inhibition of NEP and ACE inhibition revealed that the inhibition at the level of the endothelial cells, possibly as plasma ANP may be removed from the plasma via another pathway, i.e., clearance receptors (33).

Increasing the dose of omapatrilat beyond 80 mg would certainly reinforce or prolong NEP inhibition, leading to a more sustained effect on the metabolism of ANP and other substrates. It could also increase the risk of potentially severe and life-threatening adverse events, such as angioneurotic edemas (34,35). In the OCTAVE trial comparing omapatrilat to enalapril in 25,000 hypertensive patients, the overall incidence of angioedema over 24 wk was 2.17% for omapatrilat and 0.63% for enalapril (11).

Effects of Sodium Balance on Omapatrilat-Induced Changes in MAP and Plasma Renin Concentration

Our results show that in mildly sodium-depleted normotensive subjects, a single oral dose of 40 mg of omapatrilat induced a larger decrease in MAP than a single oral dose of 20 mg of fosinopril. Interestingly, the high-salt diet blunted the BP-lowering effect of F20 observed in the low-sodium status subjects but could not prevent the fall in MAP induced by omapatrilat for up to 24 h after administration. In both panels, the decrease in MAP after 40 or 80 mg of omapatrilat intake was not dose-dependent. These results are in accordance with the results obtained in the DOCA-salt model of hypertension (36) but also in sodium-sensitive hypertensive patients (10). They may have a major clinical consequence; the BP-lowering effect of omapatrilat in hypertensive patients is probably less dependent on sodium intake than that of ACE inhibitors. In the OCTAVE trial, after an 8-wk forced titration phase to reach target BP (<140/90 mmHg), omapatrilat significantly reduced systolic BP more than enalapril by 3 to 4 mmHg even though the enalapril group had more frequent increases in dosage and additions of other antihypertensive agents (11).

Several mechanisms may explain the greater BP-lowering efficacy of O40 and O80 compared with F20 in both groups despite the fact that they appear to inhibit plasma ACE similarly. An ex vivo measurement of plasma ACE activity cannot investigate the in vivo tissue ACE inhibition, and tissue ACE appears to have a much more important role in converting angiotensin I (AngI) to AngII than does plasma ACE (37). Although we did not measure plasma AngII concentrations, the though the Ki for both ACE and NEP is very similar in vitro (NEP, 8.9 nM; ACE, 6 nM), 24 h after the administration of a single oral dose of 10 mg/kg omapatrilat to normotensive rats, renal ACE was inhibited by more than 75%, whereas renal NEP was only inhibited by 30%. This profile is probably characteristic of omapatrilat. Each vasopeptidase inhibitor probably behaves in a specific way (24–26), and thus this new method offers an opportunity to compare different vasopeptidase inhibitors in humans.

Finally, contrasting with its major effects on urine ANP concentration, omapatrilat had little effect on plasma ANP concentrations, even in conditions of high-sodium intake. This is in accordance with most other studies on vasopeptidase inhibitors (13,27,28) or pure NEP inhibitors (29–32). Plasma ANP does not seem to be a sensitive marker to investigate the magnitude of NEP inhibition at the level of the endothelial cells, possibly as plasma ANP may be removed from the plasma via another pathway, i.e., clearance receptors (33).

Increasing the dose of omapatrilat beyond 80 mg would certainly reinforce or prolong NEP inhibition, leading to a more sustained effect on the metabolism of ANP and other substrates. It could also increase the risk of potentially severe and life-threatening adverse events, such as angioneurotic edemas (34,35). In the OCTAVE trial comparing omapatrilat to enalapril in 25,000 hypertensive patients, the overall incidence of angioedema over 24 wk was 2.17% for omapatrilat and 0.63% for enalapril (11).
rise in plasma active renin 24 h after 40 mg of omapatrilat was more marked than after 20 mg of fosinopril, which suggests a more important decrease in the intrarenal AngII concentrations that regulate renin release at the level of juxtaglomerular cells (38) and thus a more intense and more prolonged efficacy of omapatrilat to block the RAS.

Conversely, the greater efficacy of omapatrilat could be due to its ability to inhibit NEP, especially in tissues. However, the effect of omapatrilat on plasma ANP was small, and the increase in urine ANP was not accompanied by an additional increase in urine sodium excretion compared with fosinopril (see below). NEP is involved in the metabolism of other vasoactive peptides, such as bradykinin (39,40) and adrenomedullin (41), which were not investigated; therefore, accumulation of these peptides in the plasma or tissues may play a role in the decrease in BP. The in vivo modulation of endothelin metabolism by NEP (42,43) cannot explain the difference in BP-lowering efficacy between omapatrilat and fosinopril, according to our measurements of plasma endothelins. Omapatrilat slightly and transiently increased plasma Big-ET1 concentrations and had no effect on plasma ET1 concentrations. These results are consistent with the known effects of NEP on Big-ET1 and ET1 metabolism; NEP is a nonspecific endopeptidase that converts proendothelin to ET1 (42) and hydrolyses ET1 (44). Finally, our results are in accordance with the results reported in patients with congestive heart failure, in whom the levels of ET1 remained unchanged after oral or intravenous omapatrilat administration (45). However, measurement of endothelins in plasma is not the ideal method for investigating the effects of endothelins within the vascular wall.

Effect of Omapatrilat on Urine Sodium Excretion

In our experiment, even high doses of omapatrilat (80 mg) had the same natriuretic effect as 20 mg of fosinopril; omapatrilat only induced a slight and transient natriuretic effect, which was more easily detected in the low-sodium group than in the high-sodium group. A trend toward an increase in 24-h urine sodium excretion induced by O80 and F20 compared with placebo was observed only in the low-salt group, but the difference between groups did not achieve statistical significance in the ANOVA.

The very mild natriuretic effect of both omapatrilat and fosinopril contrasts with their major effects of BP and plasma active renin concentrations. It is consistent with previously published results on omapatrilat (10,46) and other vasopeptidase inhibitors (47). The effect of vasopeptidase inhibitors on urine sodium excretion probably depends on the clinical setting (3) or on the experimental model (48) used.

The inhibitory effect of omapatrilat on NEP and therefore its natriuretic effect might have been masked by the marked fall in BP observed in both the low- and high-salt panel groups. The preservation of a natriuresis of a magnitude similar to that of 20 mg of fosinopril by omapatrilat especially in the low-salt group, even though omapatrilat lowered BP much more and increased plasma active renin concentration more than fosinopril, may indicate per se a natriuretic effect due to the increase in urine ANP concentration after tubular NEP inhibition. In fact, the larger decrease in BP induced by omapatrilat was expected to reduce natriuresis by comparison with 20 mg of fosinopril. Morazo et al. (49) have shown that chronic treatment with omapatrilat normalizes BP in spontaneously hypertensive rats without affecting the renal ability to eliminate a sodium load by resetting the relationship between chronic pressure and natriuresis to the normal level without changing the slope of the relationship. This is the opposite of what is observed with ACE inhibitors, where the chronic pressure-natriuresis relationship is shifted to the left and is somewhat depressed (50).

In conclusion, the in vivo inhibition of ACE and NEP can be monitored by sequentially measuring urine concentrations of two specific substrates of these enzymes, AcSDKP and ANP. After a single oral dose of 40 or 80 mg of omapatrilat, ACE inhibition was more marked and persisted longer than NEP inhibition. Omapatrilat (40 mg) decreased BP more than the selective ACE inhibitor, fosinopril (20 mg), in both a low- and a high-sodium intake group. There was no major difference between the enzymatic, hemodynamic, and hormonal effects of single doses of 40 and 80 mg of omapatrilat. The BP effect of a vasopeptidase inhibitor persisted in the presence of high-sodium intake, a phenomenon that does not occur after specific ACE inhibition.

Some of the specific pharmacodynamic effects of vasopeptidase inhibition may explain its greater efficacy than ACE inhibition to reduce and control high BP levels of hypertensive patients even with low-to-normal renin levels (10), a frequently encountered situation in which ACE inhibitors are less effective. However, the different pharmacodynamic profile of vasopeptidase inhibitors may have deleterious effects. For a 3 to 4 mmHg difference in systolic BP between omapatrilat and enalapril in the OCTAVE trial, the relative risk of having a mild-to-moderate angioedema was increased by 3.4 (11).

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