Effects of Omapatrilat on the Renin-Angiotensin System in Salt-Sensitive Hypertension

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The contribution of angiotensin-(1-7) [Ang-(1-7)] to the antihypertensive actions of omapatrilat, a novel vasopeptidase inhibitor, was evaluated in 22 salt-sensitive, low renin, hypertensive subjects as a substudy of a multicenter randomized, double-blind, parallel study of 4 weeks duration. A total of 25 other subjects received lisinopril as the active control. Omapatrilat (40 mg) produced sustained control of blood pressure (BP) (as assessed by 24-h ambulatory BP measurements) that was significantly greater than that produced by 20 mg daily of lisinopril. The antihypertensive response to either drug was accompanied by similar sustained inhibition of angiotensin converting enzyme activity. Plasma levels of angiotensin I (Ang I), angiotensin II (Ang II) and Ang-(1-7) were not altered by treatment with either omapatrilat or lisinopril, even though both regimens produced a modest rise in plasma renin activity. In contrast, urinary excretion rates of Ang I and Ang-(1-7) but not Ang II increased significantly throughout the dosing period of subjects who were given omapatrilat, whereas the smaller antihypertensive response produced by lisinopril had a smaller and transient effect on increasing urinary excretion rates of Ang-(1-7). Omapatrilat, being a single molecule inhibiting neutral endopeptidase and converting enzyme simultaneously, controlled salt-sensitive hypertension by a mechanism that was associated with sustained increases in urinary Ang-(1-7) excretion. We suggest that Ang-(1-7) may be a component of the mechanisms by which omapatrilat induces an antihypertensive response in salt sensitive hypertension. Am J Hypertens 2002;15:557–564 © 2002 American Journal of Hypertension, Ltd.

Key Words: Angiotensin-(1-7), omapatrilat, lisinopril, hypertension, vasopeptidase inhibitor, salt sensitivity.

Vasopeptidase inhibitors, which are innovative drugs that combine within a single molecule inhibitory actions of the enzymatic activity of angiotensin converting enzyme (ACE) and neutral endopeptidase 24.11, may show superior efficacy in the treatment of high blood pressure (BP), especially in low-renin salt-sensitive hypertensive individuals in whom ACE inhibitors are less effective.1–3 Omapatrilat, the most clinically advanced vasopeptidase inhibitor, has similar inhibitory constants for both neutral endopeptidase 24.11 and ACE and it is effective in controlling all forms of primary essential hypertension.4,5

The antihypertensive action of vasopeptidase inhibitors is primarily the result of the combined inhibition of angiotensin II (Ang II) formation and degradation of atrial natriuretic peptide and bradykinin.4,5 However, both enzymes act on multiple substrates, one of which is the vasodilator, natriuretic, and antitrophic hormone angiotensin-(1-7) [Ang-(1-7)].5 Neutral endopeptidase 24.11 is one of three identified Ang-(1-7)–forming enzymes.7,8 In renal tissue, where tubular ACE exists in lower concentrations, neutral endopeptidase 24.11 degrades Ang-(1-7) into Ang-(1-4).9 Thus, neutral endopeptidase 24.11 may play an important role on both the synthesis and degradation of Ang-(1-7). Furthermore, neutral endopeptidase 24.11 can cleave Ang II into Ang-(1-4).10 These findings raised the question as to whether a chemical inhibitor of both ACE and neutral endopeptidase 24.11 could have a significant effect on the activity of Ang-(1-7) and its contribution to BP regulation, a possibility underscored by the observa-
tion in both humans and animals that hypertension is associated with reduced production and activity of the heptapeptide.\(^{11,12}\)

We conducted a multicenter, randomized, double-blind, parallel-design study focusing on comparing the effect of omapatrilat and lisinopril on the BP and urinary excretion of atrial natriuretic peptide in salt-sensitive hypertensive subjects to assess the effect of omapatrilat on the urinary excretion rates of angiotensin I (Ang I), angiotensin II (Ang II), and Ang-(1-7). Previous studies in human subjects and hypertensive animals showed that their rates of excretion in urine provided accurate reflections of the rate of synthesis, metabolism, and action of angiotensin peptides in the kidney.\(^{10}\)

### Patients and Methods

This study was performed in 47 of 167 subjects who were enrolled to participate in a multicenter, double-blind study evaluating the efficacy of either omapatrilat or lisinopril in controlling the arterial pressure of salt-sensitive hypertensive subjects. Other clinical and hormonal findings, including the effects of the therapies on plasma and urine electrolytes, plasma aldosterone, and plasma and urine concentrations of both atrial natriuretic peptide and cyclic guanosine monophosphate are reported elsewhere.\(^{13}\)

### Study Population

The study enrolled patients with stage I and II hypertension (seated diastolic blood pressure [SeDBP] 95 to 110 mm Hg), who were 18 to 78 years of age. Both men and women (not nursing, pregnant, or of childbearing potential) were included. The clinical trial was approved by the contributing Institutional Review Boards and performed in accordance with the principles of the Declaration of Helsinki and its amendments. All patients provided written informed consent.

### Study Design

After a 2- to 3-week, single-blind, placebo lead-in period, 47 subjects had their salt-sensitivity status evaluated based on a decrease in mean arterial pressure of ≥10 mm Hg after sodium and volume depletion compared with the end of the saline infusion.\(^{14}\)

### Table 1. Demographic characteristics of patients randomized to treatment groups

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>(n = 22)</th>
<th>(n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td>(52 \pm 9)</td>
<td>(54 \pm 1)</td>
</tr>
<tr>
<td><strong>Sex, (n)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td><strong>Ethnicity, (n)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>African American</strong></td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td><strong>White</strong></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td><strong>Weight, kg</strong></td>
<td>(84 \pm 16)</td>
<td>(89 \pm 18)</td>
</tr>
</tbody>
</table>

Data are mean ± SD.

Age, sex, and ethnicity data are reported in Table 1.

A total of 22 salt-sensitive hypertensive subjects were randomized to receive omapatrilat 10 mg/day for 1 week followed by 3 weeks of 40 mg/day of omapatrilat. The remaining subjects \((n = 25)\) received lisinopril 10 mg/day for the first week, followed by 20 mg for the next 3 weeks. The doses chosen were within each drug’s respective therapeutic range. Angiotensin peptide data from one of the 22 subjects in the omapatrilat-treated group and one from the lisinopril-treated group, were lost because of improper handling and processing; therefore, the data reported here are from 21 patients randomized to omapatrilat and the 24 other given lisinopril.

A primary objective of our substudy was to compare the effects of omapatrilat and lisinopril on the plasma levels and urinary excretion of angiotensin peptides. Secondary variables included changes in 24-h average ambulatory BP and measurements of plasma ACE activity.

### Hormonal Measurements

The 24-h urine samples were collected before and after treatment to measure volume, creatinine, and urinary excretion of angiotensin peptides. Separate urinary samples were obtained between 0 and 4 h, 4 and 8 h, 8 and 12 h, 12 and 20 h, and 20 and 24 h before dosing (day 1) and after the last dosing of either drug (day 28). Venous blood was collected in Vacutainer tubes (Becton Dickinson, Franklin, NJ) containing K\(_2\) EDTA (as an anticoagulant) and aprotonin. Blood samples for ACE activity contained heparin, whereas those used for the assay of angiotensin peptides were collected in a tube containing a mixture of peptidase inhibitors.\(^{11}\) Blood samples were collected before as well as 4 h after administration of the first dose of either omapatrilat or lisinopril (day 1) and after 4 weeks (day 28). Urine collections were obtained in plastic jars. Plasma and urinary concentrations of angiotensin peptides and plasma renin activity were processed for radioimmunoassay, as previously described in detail.\(^{11}\)
Blood Pressure Measurements

Ambulatory blood pressure monitoring (ABPM) was performed under similar circumstances (ie, same day of week and time of day, starting ≥1 h before study drug administration) and using similar devices (SpaceLabs model 90207; SpaceLabs, Redmond, WA) precalibrated by the manufacturer and validated by mercury sphygmomanometer before each initiation using the nondominant arm. A recording period of 25 h covered the trough phase on the morning after device attachment. Subjects did not take the next dose until the ABPM device was removed. An ABPM recording was considered adequate for analysis if the recording period was ≥24 h, there was at least one valid reading hourly, and ≥75% of the programmed readings remained after all editing were completed. If a recording failed to meet these criteria, it was repeated. Cuff BP measurements were obtained from the dominant arm using a standard mercury manometer with an appropriate sized cuff applied to the mid arm centered over the brachial artery (regular-sized cuff for arms measuring 24 to 32 cm in circumference, and large cuff for arms 32 to 41 cm in circumference). If the average of three simultaneous ABP measurements and adjusted mercury manometer readings (for systolic BP, 4 mm Hg was added to the first sounds heard; for DBP, 4 mm Hg was subtracted from the last sounds heard) did not agree within 5 mm Hg, the cuff was repositioned. The device was reinitialized if more than six readings had been stored without achieving agreement within 5 mm Hg.

Statistical Methods

All analyses were carried out using SAS/STAT version 6.08 (SAS Institute, Cary, NC). Student t test for continuous variables was used, and a value of $P < 0.05$ was set for statistical significance.

Results

There were no differences in age, sex, body weight (Table 1), and body mass index among the salt sensitive subjects randomized to either omapatrilat or lisinopril treatments. Both study groups had a larger representation of African American compared with white subjects.

Baseline 24-h ambulatory systolic and diastolic BPs averaged 147 ± 5/90 ± 4 mm Hg (mean ± SD) in patients before randomization to lisinopril 20 mg daily and 148 ± 5/94 ± 5 mm Hg in those assigned to omapatrilat 40 mg daily. Average 24-h baseline heart rate averaged 73 ± 3 beats/min and 76 ± 4 beats/min in salt-sensitive patients randomized to either lisinopril or omapatrilat, respectively. Differences in baseline values between the two groups were not statistically significant ($P > 0.05$).

In the group receiving omapatrilat, 24-h mean (±SD) arterial pressure averaged 112 ± 5 mm Hg before (day 1) and 101 ± 5 mm Hg at day 28, a difference of −11 ± 1.45 mm Hg ($P < .001$). In subjects randomized to the lisinopril treatment arm, 24-h baseline mean arterial pressure was 110 ± 5 mm Hg at day 1 and 103 ± 5 mm Hg at day 28, a mean difference change of −6.18 ± 1.34 mm Hg ($P < .001$). The difference in mean arterial pressure between the two treatment arms at the end of the study (day 28, −3 ± 1 mm Hg) was statistically significant at $P < .03$ and associated with no changes in heart rate. The time course of the hourly intervals in mean arterial pressure at baseline and day 28 are illustrated in Fig. 1.

Plasma Renin Activity, Angiotensin Converting Enzyme, and Angiotensin Peptides

Baseline plasma renin and ACE activities were not different in the subjects randomized to either omapatrilat or
Angiotensin peptides were found with regularity in the urine of all subjects. For the group as a whole (n = 46), urinary concentrations of Ang I, Ang II, and Ang-(1-7) averaged 226 ± 34 fmol/mL, 69 ± 17 fmol/mL, and 260 ± 19 fmol/mL, respectively. Similar to previous findings, the concentrations of Ang I and Ang-(1-7) in urine were 598% and 1060% higher, respectively, than those determined for plasma.

Table 3 shows the average 24-h urinary excretion rates of the angiotensins both before and at 28 days of treatment with either omapatrilat or lisinopril. The 24-h urinary excretion rates of Ang-(1-7) but not Ang I and Ang II were significantly elevated in subjects randomized to omapatrilat. Although the average 24-h excretion rate of urinary Ang-(1-7) increased 2.34-fold in subjects given omapatrilat, it increased by only 18% in those given lisinopril. Figs. 2 and 3 illustrate the time course of the changes in the urinary excretion patterns of angiotensin peptides before and at day 28 in subjects randomized to either omapatrilat or lisinopril. Urinary Ang I excretion was significantly higher in omapatrilat-treated subjects during the first 8 h postdose and did not change in subjects given lisinopril. Furthermore, omapatrilat treated subjects had significantly higher rates of urinary excretion of Ang-(1-7) that were sustained for the entire 24 h postdose (Fig. 2). In contrast, in subjects given lisinopril urinary excretion rates of Ang-(1-7) increased only between 8 and 20 h postdosing (Fig. 3). Neither of the two inhibitors had an effect on urinary rates of Ang II excretion.

Discussion

Dual vasopeptidase inhibitors are a new class of antihypertensive agents that have proved effective in controlling BP. Their mechanism of action entails both inhibition of Ang II synthesis and reduced metabolism of atrial natriuretic peptide. A prospective, double-blind, randomized study comparing omapatrilat and lisinopril in salt-sensitive hypertensive individuals13 afforded us the opportunity to assess the effect of the vasopeptidase inhibitor on angiotensin peptide concentrations and urinary excretion. Omapatrilat demonstrated superiority over lisinopril in controlling the arterial pressure of salt-sensitive hypertensive subjects, providing further evidence for a
difference in the mechanisms that contribute to the maintenance of high BP in this form of essential hypertension.

Salt sensitivity was evaluated in each of the subjects using a short-term protocol that cycled subjects from a state of acute sodium volume loading to one of sodium and volume depletion.\textsuperscript{18} The subgroup of patients randomized to either omapatrilat or lisinopril and part of the current groups of subjects enrolled in the primary study\textsuperscript{13} in terms of their demographics variables or BP and heart rate outcomes. As in the larger study, a greater efficacy of omapatrilat over lisinopril was demonstrated by 24-h recordings of ambulatory BP.

In the primary study,\textsuperscript{13} the greater antihypertensive effect of omapatrilat over lisinopril was associated with 3.8-fold increases in the urinary excretion of atrial natriuretic peptide and cGMP, both markers of effective inhibition of neutral endopeptidase 24.11. No similar changes were found in the salt-sensitive subjects receiving lisinopril. Our data now showed that the increase in urinary excretion of atrial natriuretic peptide is associated with a significant and differential effect of the two drugs on renal excretion rates of Ang-(1-7). Urinary excretion of Ang-(1-7) showed a 2.34-fold increase in salt sensitive subjects randomized to omapatrilat, a finding that further contrasted with the clearly smaller, delayed, and transient 18% peak rise in urinary Ang-(1-7) in patients given lisinopril.

Although both agents effectively inhibited plasma ACE activity, the degree of inhibition was higher for those subjects randomized to lisinopril. Plasma renin activity rose in response to omapatrilat and lisinopril, but in both cases the augmentation in renin activity was smaller than anticipated.\textsuperscript{19,20} In contrast, plasma angiotensin peptide levels remained essentially unchanged with respect to baseline values and did not differ significantly between subjects treated with either omapatrilat or lisinopril. The failure of either drug to cause the expected pattern of changes in plasma levels of Ang I and Ang II in the phase of a sustained inhibition of ACE\textsuperscript{21–23} is a potentially important finding. The uncoupling between ACE inhibition and the circulating angiotensin system provides further evidence for a disturbance in the biochemical mechanisms that regulate the role of the renin-angiotensin system in salt-sensitive hypertension. In keeping with this interpretation, plasma renin activity levels were in the lower range of normal, supporting the fact that salt-sensitive subjects comprised a low-renin group. In addition, the increases in plasma renin activity achieved with either omapatrilat or lisinopril were substantially less than those reported in previous studies in non–salt sensitive hypertensive individuals.\textsuperscript{24,25}

Although chronic exposure to omapatrilat or lisinopril had no effect on the circulating components of the renin-angiotensin system, measurements of angiotensin peptides in urine demonstrated a clearly differential effect. Baseline 24-h urinary excretion rates of the three angiotensin peptides only a

\begin{table}
\centering
\caption{Twenty-four-hour urinary excretion rates}
\begin{tabular}{lrr}
\hline
 & \multicolumn{2}{c}{Treatment Group} \\
 & Omapatrilat & Lisinopril \\
 & \textit{(n = 21)} & \textit{(n = 24)} \\
\hline
Angiotensin I, pmol/24 h & & \\
Day 1 & 84.08 ± 9.71 & 90.58 ± 9.96 \\
Day 28 & 103.04 ± 13.12 & 94.25 ± 12.26 \\
\textit{P} value & >.05 & \\
Angiotensin II, pmol/24 h & & \\
Day 1 & 20.05 ± 6.11 & 31.46 ± 8.92 \\
Day 28 & 18.72 ± 3.64 & 23.47 ± 5.50 \\
\textit{P} value & >.05 & \\
Angiotensin-(1-7), pmol/24 h & & \\
Day 1 & 100.80 ± 10.88 & 101.37 ± 6.68 \\
Day 28 & 235.71 ± 30.29* & 119.70 ± 6.54 \\
\textit{P} value & >.05 & \\
\hline
\end{tabular}
\end{table}

Data are mean ± SE.
\textit{P} values denote differences between values in subjects treated with either omapatrilat or lisinopril.

* \textit{P} < .001 v day 1.

\textsuperscript{561}
that the changes observed in the urine were not due to increased renal filtration of the peptides from the plasma. We have reported that urinary excretion rates of Ang-(1-7) are significantly reduced in untreated essential hypertensive subjects, averaging 60 pmol/24 h compared with 99 pmol/24 h in normotensive volunteers.\textsuperscript{11} Urinary excretion rates of Ang-(1-7) in the current studies are similar to those found in normotensive subjects,\textsuperscript{11} a finding that

\begin{figure}[h!]
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Values are means $\pm$ SEM of urinary excretion of angiotensin peptides in subjects randomized to omapatrilat (40 mg) on day 1 (open bars) and on last day of regimen (day 28, solid bars). With the exception of data reported for the interval 12 to 20 h, all other values are from 4-h urinary collections. *$P < 0.05$ v corresponding baseline values obtained during first dose of agent on day 1 (open bars).}
\end{figure}

\begin{figure}[h!]
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{Values are means $\pm$ SEM of the urinary excretion of angiotensin peptides in subjects randomized to lisinopril (10 mg) on first day (day 1, open bars) and on last day (day 28, solid bars) of regimen. With the exception of data reported for the interval 12 to 20 h, all other values are from 4-h urinary collections. *$P < 0.05$ v corresponding baseline values obtained during first dosing of agent on day 1 (open bars).}
\end{figure}
might suggest a difference between salt-sensitive and non-salt-sensitive hypertensive subjects. Although the previous study was done in a white population, the greater proportion of African American subjects in the current study does not exclude a possible effect of ethnicity on these findings.

Experimental studies on the mechanisms of Ang-(1-7) formation and metabolism showed that neutral endopeptidase 24.11 is one of several Ang-(1-7)-forming enzymes.28 Both ACE30 and an N-terminal derived form of ACE in urine31 converts Ang-(1-7) into Ang-(1-5).32 However, in the kidney, neutral endopeptidase 24.11 degrades Ang-(1-7) into Ang-(1-4).28 In keeping with these findings, we have reported that omapatrilat inhibits the metabolism of Ang-(1-7) in urine.10 The multiple enzymatic pathways that could contribute to the formation and metabolism of Ang-(1-7) are exemplified by the demonstration that a novel ACE-related carboxypeptidase (ACE2)33 an oligopeptidase B34 and a postproline carboxypeptidase (angiotensinase C)35 converts Ang II into Ang-(1-7). Therefore, substrate availability in relation to tissue enzyme compartmentalization may determine the action of tissue endopeptidases on the metabolism and synthesis of angiotensin peptides.

The vasodilator effect of omapatrilat has been attributed to an increase in the activity of atrial and brain natriuretic peptides, bradykinin, and adrenomedullin, in combination with suppression of Ang II formation.13 In this study we found that the increases in both urinary excretion of the atrial natriuretic peptide and cGMP were not sustained for >12 h, in contrast to the larger and sustained rise in urinary Ang-(1-7) excretion throughout the 24-h postdosing period. Although these data do not minimize the importance of increased levels of atrial natriuretic peptide in contributing to the mode of action of the vasopeptidase inhibitor, an additional contribution of Ang-(1-7) to the vasodilator effects of omapatrilat is suggested (but obviously not proved) by our studies. Further work employing selective antagonists of Ang-(1-7), bradykinin, and natriuretic peptides is required to ascertain the relative contributions and interactions of vasodilator mechanisms in mediating the antihypertensive effect of omapatrilat in salt-sensitive hypertension.

In summary, omapatrilat is an effective antihypertensive agent for use in individuals with a greater sensitivity to volume and salt expansion. The greater potency of omapatrilat appears not to be related entirely to inhibition of ACE or to suppression of circulating Ang II. Our findings suggest an additional contribution of Ang-(1-7) to the vasodilator effects of the inhibitor, an interpretation that is not meant to exclude an important role of atrial natriuretic peptide and bradykinin to the overall hemodynamic and renal effects of omapatrilat. We further suggest that the large increases in the urinary excretion of Ang-(1-7) after omapatrilat treatment may result from the combined inhibition of two major metabolic pathways participating in the degradation of the peptide in the renal tubules.

The dissimilar effects of lisinopril on urinary excretion of Ang I and Ang-(1-7) and atrial natriuretic peptide13 suggest a dysregulation of the biochemical factors contributing to BP control by the renin-angiotensin system in salt sensitive hypertensive subjects.

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References


