Renal Hemodynamic and Natriuretic Effects of Concomitant Angiotensin-Converting Enzyme and Neutral Endopeptidase Inhibition in Men

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Abstract—This double-blind placebo-controlled study was designed to investigate the acute and sustained hormonal, renal hemodynamic, and tubular effects of concomitant ACE and neutral endopeptidase (NEP) inhibition by omapatrilat, a vasopeptidase inhibitor, in men. Thirty-two normotensive subjects were randomized to receive a placebo, omapatrilat (40 or 80 mg), or the fosinopril/hydrochlorothiazide (FOS/HCTZ; 20 and 12.5 mg, respectively) fixed combination for 1 week. Blood pressure, renal hemodynamics, urinary electrolytes and atrial natriuretic peptide excretion, and several components of the renin-angiotensin system were measured for 6 hours on days 1 and 7 of drug administration. When compared with the placebo and the FOS/HCTZ combination, omapatrilat induced a significant decrease in plasma angiotensin II levels (P<0.001 versus placebo; P<0.05 versus FOS/HCTZ) and an increase in urinary atrial natriuretic peptide excretion (P<0.01). These hormonal effects were associated with a significant fall in blood pressure (P<0.01) and a marked renal vasodilatation, but with no significant changes in glomerular filtration rate. The FOS/HCTZ markedly increased urinary sodium excretion (P<0.001). The acute natriuretic response to FOS/HCTZ was significantly greater than that observed with omapatrilat (P<0.01). Over 1 week, however, the cumulative sodium excretion induced by both doses of omapatrilat (P<0.01 versus placebo) was at least as great as that induced by the dose of FOS/HCTZ (P=NS versus FOS/HCTZ). In conclusion, the results of the present study in normal subjects demonstrate that omapatrilat has favorable renal hemodynamic effects. Omapatrilat combines potent ACE inhibition with a sustained natriuresis, which explains its well-documented potent antihypertensive efficacy. (Hypertension. 2002;40:1112-1120.)

Key Words: hemodynamics, renal ■ sodium ■ angiotensin-converting enzyme ■ neutral endopeptidase ■ omapatrilat ■ vasopeptidase ■ human

Angiotensin II is an important physiological modulator of renal function through its hemodynamic, glomerular, and tubular effects. Hence, under most circumstances, interruption of the renin-angiotensin cascade with ACE inhibitors or angiotensin II type 1 receptor antagonists in humans promotes sodium excretion and increases renal blood flow without affecting glomerular filtration rate (GFR). As a result, filtration fraction decreases. Atrial natriuretic peptide (ANP), the brain natriuretic peptide, and the C-type natriuretic peptide represent another family of peptides that have an important impact on renal function. Indeed, in addition to a peripheral vasodilatation, these peptides elicit diuresis and natriuresis, an attenuation of the release of renin and aldosterone and of the sympathetic nervous activity. High plasma ANP and brain natriuretic peptide levels have been measured under several clinical conditions in which sodium and water retention occur, such as congestive heart failure or chronic renal failure. In humans, the administration of exogenous ANP and the inhibition of natriuretic peptide degradation by use of neutral endopeptidase inhibitors have been found to increase sodium and water excretion and to lower blood pressure.

Omapatrilat is a member of the new drug class of vasopeptidase inhibitors that possess the ability to inhibit the membrane-bound zinc metalloproteases ACE EC 2.4.15.1 and the neutral endopeptidase EC 3.4.24.11 (NEP). Thus, omapatrilat simultaneously decreases angiotensin II generation by inhibiting ACE activity and reduces the metabolic degradation of natriuretic peptides by inhibiting NEP. In experimental hypertension, omapatrilat has been found to lower blood pressure in low-, normal-, and high-renin hypertension models and in humans, preliminary dose-finding studies have shown that omapatrilat reduces blood pressure dose-dependently at doses ranging between 5 and 80 mg. Experimental and preliminary clinical studies also suggest that omapatrilat has favorable effects in heart failure.

Because of its ability to inhibit angiotensin II generation and to prevent the degradation of natriuretic peptides, oma-
The volunteers were studied on a fixed-sodium diet to achieve a baseline systolic and diastolic blood pressures were 118 ± 6 mmHg. Thirty-two healthy male volunteers, age 19 to 32 years (mean, 25 years) were randomized to receive for 7 days a placebo, omapatrilat (40 or 80 mg), or an ACE inhibitor/diuretic combination (fosinopril 20 mg and hydrochlorothiazide 12.5 mg) according to a double-blind parallel-group study design.

Methods

The volunteers were studied on a fixed-sodium diet to achieve a baseline sodium excretion of ~150 mmol/d. The diet was begun 3 days before drug administration and was maintained during the next 7 treatment days. The diet was provided under the supervision of a dietitian in the hospital, where the subjects ate all their meals. Diet compliance was monitored by repeated 24-hour urine collections. Caffeine containing beverages were strictly forbidden during the diet. The nature and purpose of the study had been explained, and written informed consent had been obtained from each subject. The protocol was approved by the hospital ethics committee.

Study Design

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On day 1 (after 3 days of diet), the volunteers came to the hospital at 7:00 AM after an overnight fast to undergo renal clearance studies as described previously.17 Volunteers were studied in supine position (except for voiding). They received a light breakfast on arrival and a snack 1 hour before drug intake. They fasted thereafter. Two intravenous catheters were inserted into antecubital veins, one for the infusion of insulin and p-aminohippuric acid (PAH) in a glucose/saline solution and a second into the contralateral forearm for blood drawing.

Between 7:00 and 8:00 AM, the volunteers received an oral water load of 400 mL. After a priming dose, the intravenous infusion of insulin and PAH was started. A fixed amount of water (150 mL/h) was given orally to sustain urine output. After a 2-hour equilibration period, two 1-hour baseline measurements were performed. At the end of the baseline periods, the subjects were randomized to receive, in a double-blind fashion, a placebo (n = 8), 40 mg omapatrilat (n = 8), 80 mg omapatrilat (n = 8), or 20 mg fosinopril combined with 12.5 mg hydrochlorothiazide (FOS/HCTZ).

Blood pressure, heart rate, urinary electrolyte excretion, and clearances of insulin and PAH were measured every 2 hours after drug intake. Plasma renin activity (PRA), plasma aldosterone, plasma angiotensin II levels, and plasma ACE activity were measured immediately before and 2 and 6 hours after drug intake. Urinary ANP were measured in all urine collections. On study days 2 to 7, the doses of placebo, omapatrilat, or FOS/HCTZ were administered every morning between 7:00 and 8:00 AM. Blood pressure and heart rate were measured with subjects in the supine position before the drug was given. Twenty-four-hour urine collections were repeated every day during treatment. On day 7, renal clearances and hormonal measurements were repeated as on day 1.

Drugs and Chemicals

Omapatrilat, FOS/HCTZ fixed combination, and placebo were provided by Bristol Myers Squibb. Inulin (Inutest, sodium salt of PAH), from Biologische Arbeitsgemeinschaft GmbH.

Analytic Methods

Plasma and urinary insulin, PAH, and electrolyte concentrations were determined as published previously.17 Aldosterone was measured by a direct radioimmunoassay.18 PRA 19 ACE activity,20 plasma angiotensin II levels,21 and urinary ANP22 were measured by use of radioimmunoassays as described previously.

Calculation of Renal Parameters and Statistical Evaluation

Clearances were calculated by the traditional method using the formula Cx = Ux × V/Px, where Ux and Px represent urine and plasma concentrations, respectively, of x, and V is the urine flow rate in milliliters per minute. Filtration fraction was calculated as the ratio of GFR to renal blood flow. All results are expressed as mean ± SEM. The statistical significance of differences was evaluated by ANCOVA for repeated measurements, with a value of P < 0.05 as the minimum level of significance. We tested for differences between and within groups. The number of subjects was calculated to demonstrate changes in renal plasma flow or GFR > 20% with a 95% confidence.

Results

After 3 days of diet, the 4 groups of subjects were comparable in terms of age, body weight, blood pressures, and heart rate. The mean values of the last two 24-hour urinary sodium excretion levels before day 1 were 159 ± 10 mmol/d in the placebo group, 158 ± 11 mmol/d in the 40-mg omapatrilat, 142 ± 11 mmol/d in the 80-mg omapatrilat group, and 158 ± 12 mmol/d in the FOS/HCTZ group (P = NS). There were no biochemical or hormonal differences between the groups at baseline. Both omapatrilat and FOS/HCTZ were well tolerated, and no significant clinical or laboratory side effects were observed.

Short-Term and Sustained Hormonal Effects

Figure 1 shows the effects of treatment with omapatrilat or FOS/HCTZ on ACE and plasma renin activities and on plasma angiotensin II and aldosterone levels at days 1 and 7. On day 1, both doses of omapatrilat and FOS/HCTZ induced a significant inhibition of ACE activity associated with a significant decrease in circulating angiotensin II levels and a reactive rise in PRA, whereas no change was found in the placebo group. A slight but nonsignificant decrease in plasma aldosterone was also found with both agents. On day 7, ACE activity was already decreased before administration of the drugs except in the placebo group. Interestingly, plasma angiotensin II levels were significantly higher at baseline in the FOS/HCTZ group than in the other 3 groups. To a lesser degree, the same pattern was found with aldosterone. At 2 hours on day 7, omapatrilat induced significantly greater decreases in ACE activity and plasma angiotensin II levels and increases in PRA than did placebo and FOS/HCTZ. At 6
hours, the decreases in angiotensin II induced by the 2 doses of omapatrilat were still significantly greater than those observed with FOS/HCTZ. Omapatrilat also induced a significant increase in urinary ANP excretion (Table 1), with the maximal effect observed 4 hours after drug intake. On day 7, baseline urinary ANP excretion was higher in all groups, suggesting that the volunteers ate more salt. Yet, similar changes in urinary ANP excretion were found.

**Short-Term and Sustained Systemic and Renal Hemodynamic Effects**

Figure 2 shows the changes in systolic and diastolic blood pressure observed on days 1 and 7. On both days, significant decreases in blood pressure were found with the 2 doses of omapatrilat. Mean systolic and diastolic blood pressures were lower in both omapatrilat groups compared with the FOS/HCTZ group at most time-points and at almost all time-points compared with placebo. On day 7, baseline blood pressures were significantly lower than on day 1 with the 3 active treatments, and further reductions were obtained with the administration of omapatrilat and FOS/HCTZ. No significant changes in heart rate were measured during the study, in particular no reflex tachycardia.

No significant change in GFR was found with the 3 active treatments. However, effective renal plasma flow increased markedly and significantly with both doses of omapatrilat (Figure 3). On days 1 and 7, all values measured after drug administration were significantly higher on omapatrilat than on placebo. The FOS/HCTZ induced only modest increases in effective renal plasma flow. The renal vasodilatation with no change in GFR resulted in a significant reduction of filling fraction with both doses of omapatrilat (Figure 3). Similar changes were found on days 1 and 7.

**Short-Term and Sustained Effects on Fluid and Sodium Excretion**

On days 1 and 7, the FOS/HCTZ association induced a marked acute increase in urinary sodium and potassium excretions, and urinary volume excretion was higher with the combination than with the placebo group (Table 1).
Although in the placebo and FOS/HCTZ groups natriuresis was equal to that of day 1, the omapatrilat-treated groups exhibited still more natriuresis on day 7 than on day 1, suggesting that subjects had still not reached a compensatory state (Table 2). The 2 doses of omapatrilat also increased urinary water and sodium excretion when compared with that of placebo, but the effect was less pronounced. In contrast to FOS/HCTZ, omapatrilat had no effect on urinary potassium excretion. Figure 4 shows the cumulative 6-hour sodium excretion in the various groups on days 1 and 7, and Figure 5 presents the cumulative sodium excretion over 1 week in the 4 groups. At 1 week, both doses of omapatrilat significantly increased urinary sodium excretion \((P<0.01\) versus placebo), and the cumulative 1-week effect was at least as important as the one obtained with the FOS/HCTZ combination.
This is the first study exploring specifically the renal hemodynamic and tubular effects of the vasopeptidase inhibitor omapatrilat in humans in comparison with an ACE inhibitor-diuretic combination. Our results demonstrate the following points: (1) at the dose of 40 and 80 mg, omapatrilat is a potent ACE inhibitor that produces greater acute and sustained decreases in circulating angiotensin II levels than does the FOS/HCTZ combination used. In addition, omapatrilat increases urinary ANP excretion, and (2) in normotensive subjects, omapatrilat lowers blood pressure and increases markedly effective renal plasma flow without affecting the GFR. These effects are more pronounced with 40 and 80 mg omapatrilat than with the FOS/HCTZ combination, and (3) omapatrilat has a modest acute natriuretic effect that is significantly less pronounced than that observed with the FOS/HCTZ combination. However, over 1 week, the cumulative sodium excretion induced by the 2 doses of omapatrilat was at least as important as that of FOS/HCTZ.

**Hormonal Effects**

Omapatrilat belongs to the new class of vasopeptidase inhibitors that simultaneously inhibit ACE and NEP. The results of the present study confirm that omapatrilat used at the doses of 40 and 80 mg daily is a potent ACE inhibitor that significantly reduces circulating angiotensin II levels, both acutely and during repeated administration, and increases urinary ANP levels. On days 1 and 7, the omapatrilat-induced blockade of the renin-angiotensin system was actually greater than that obtained with 20 mg fosinopril, which is a dose of fosinopril commonly recommended for the treatment of hypertension. Yet, one has to take into account that only 1 dose of fosinopril was investigated.

Thus, one cannot exclude that higher doses would lead to a comparable ACE inhibition. Of particular interest are the plasma angiotensin II levels measured at trough on day 7. In the omapatrilat groups, plasma angiotensin II levels were comparable before drug intake on days 1 and 7. This suggests that ACE inhibition with omapatrilat does not persist around the clock, although these results have to be assessed in relation with the substantial cumulative natriuresis that takes place between days 1 and 7. However, in the FOS/HCTZ group, plasma angiotensin levels were significantly increased at trough on day 7 compared with the baseline value of day 1. This is also owing to the diuretic effect of the thiazide, which leads to a sodium loss and a compensatory activation of the renin-angiotensin system. In this respect, a similar pattern was observed with aldosterone. It is, however, important to notice that although the FOS/HCTZ combination reduces circulating angiotensin II levels on days 1 and 7, the circulating angiotensin II levels achieved during repeated administration of the ACE inhibitor/diuretic combination are far from being suppressed, even at 2 and 6 hours after drug intake.

We have reported previously that NEP inhibition results in an increase in urinary ANP and cGMP levels with little, if any, changes in plasma ANP and cGMP concentrations. Accordingly, a higher urinary ANP excretion was found with the 2 doses of omapatrilat. This effect of omapatrilat was not observed in the placebo group or in the FOS/HCTZ group. The time course of the omapatrilat-
induced NEP inhibition suggests that the drug does not inhibit the NEP enzyme throughout the day. However, in salt-sensitive hypertensive patients, a slight but significant increase in plasma ANP was found with omapatrilat when compared with lisinopril.24 Nevertheless, after 1 month of treatment, urinary ANP excretion during the last 4 hours of the day was not significantly different in the lisinopril and omapatrilat groups.

Systemic and Renal Hemodynamic Effects
Several clinical studies have demonstrated that combined ACE/NEP inhibition is an effective way to reduce blood pressure in hypertensive patients.12,13,25 Preliminary investigations conducted with omapatrilat have shown that this compound effectively lowers blood pressure.12,13 In accordance with these observations, we found that even though the study was conducted in normotensive subjects, both doses of omapatrilat lowered blood pressure, and the effect of the 80 mg dose was significantly greater than that obtained with the FOS/HCTZ combination. The fall in blood pressure induced by omapatrilat was dose-dependent and persisted during repeated administration. Using another NEP/ACE inhibitor (MDL 100,240), Roussos et al26 found a significant decrease in systolic blood pressure only in moderately salt-depleted normotensive subjects. In the present study, subjects received a normal sodium intake, ie, 150 mmol of sodium per day, and blood pressure was measured only in supine position. The changes in blood pressure could have been even more marked in standing position.

Blockade of the renin-angiotensin system with ACE inhibitors or angiotensin II receptor antagonists usually results in an increase in effective renal plasma flow with no change in GFR; hence, filtration fraction decreases.2 In contrast, the administration of exogenous ANP produces no change in GFR and little or no change in effective renal plasma flow.5 Whether dual NEP/ACE inhibitors produce a renal vasodilation and changes in GFR is less clear. Indeed, some renal vasodilation has been reported in animal models of heart failure treated with omapatrilat.16 However, these changes could have been attributed to the improvement in cardiac output. In normotensive subjects, MDL 100,240 had no effect on GFR and renal plasma flow.26 In the present study, omapatrilat induced a marked dose-dependent increase in effective renal plasma flow with no change in GFR. This resulted in a marked decrease in filtration fraction, in particular with the highest dose of omapatrilat. Thus, the pattern of changes in renal hemodynamics obtained with the administration of omapatrilat is very similar to that of a potent ACE inhibitor. Yet, the renal hemodynamic effect of omapatrilat could also be mediated by the local increase in natriuretic peptides, including urodilatin within the kidney. The renal hemodynamic profile of omapatrilat may be very beneficial in terms of renal protection as it enables to lower intraglomerular pressure as reflected by the fall in filtration fraction. In this respect, recent experimental studies have demonstrated that omapatrilat has a favorable impact on the progression of chronic renal failure.27,28

Tubular Effects
Although in experimental studies, it was found that omapatrilat increases sodium excretion,16,28 the importance of this natriuretic response has often been downplayed as it was considered a marginal effect documented almost exclusively in heart failure models. One goal of the present study was to compare the natriuresis obtained with omapatrilat with that achieved with an ACE inhibitor/diuretic combination. The choice of the fixed combination was to compare omapatrilat, which combines 2 effects, with one very popular fixed combination that includes an ACE inhibitor. Our results clearly demonstrate that omapatrilat has some natriuretic properties. However, the natriuretic response is not dose-dependent, probably because blood pressure falls with the highest dose of omapatrilat and, hence, blunts the natriuretic effect of the drug. Our data also show that the acute omapatrilat-induced sodium excretion is quantitatively less important than that obtained with FOS/HCTZ. However, over 1 week, the increase in sodium excretion compared with the placebo group is at least as important as the one induced by FOS/HCTZ. This suggests that the natriuretic property of omapatrilat is acutely less important but perhaps more sustained during the day, a profile that may explain the long-term effect on sodium balance.

One important question was the following: does the natriuretic response to omapatrilat contribute to the fall in blood pressure? In our subjects, omapatrilat produces an acute fall in blood pressure at a time when the natriuretic response is still modest, as illustrated in Figure 6, which shows the relationships between the acute changes in blood pressure and the cumulative 6-hour sodium excretion (right panel) or the change in circulating angiotensin II (left panel) on day 1. It is evident from this representation that the acute fall in blood pressure in the omapatrilat group is at least as important as the one induced by FOS/HCTZ. However, the acute natriuretic response is not dose-dependent, probably because blood pressure falls with the highest dose of omapatrilat and, hence, blunts the natriuretic effect of the drug. Our data also show that the acute omapatrilat-induced sodium excretion is quantitatively less important than that obtained with FOS/HCTZ. However, over 1 week, the increase in sodium excretion compared with the placebo group is at least as important as the one induced by FOS/HCTZ. This suggests that the natriuretic property of omapatrilat is acutely less important but perhaps more sustained during the day, a profile that may explain the long-term effect on sodium balance.

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pressure obtained with omapatrilat relates primarily to the suppression of circulating angiotensin II rather than to the natriuretic response. The greater effect on blood pressure obtained with omapatrilat compared with FOS/HCTZ may reflect the additional NEP inhibition, but it may also be owing to the more complete ACE inhibition and ensuing angiotensin II reduction. Yet, this relationship does not exclude that the omapatrilat-induced natriuresis contributes to the long-term blood pressure-lowering effect of this compound. Indeed, the effect of omapatrilat on the 1-week cumulative sodium excretion is pronounced and equivalent to that of FOS/HCTZ. Thus, this natriuresis probably contributes to the long-term antihypertensive efficacy of the compound. Furthermore, one has to emphasize that the natriuresis is not completely abolished by the fall in blood pressure. This preserved capacity to excrete sodium when blood pressure is low may be of particular interest in some clinical conditions such as congestive heart failure. In this context, omapatrilat has recently been found to offer a greater improvement in New York Heart Association class and reduction in combined mortality/hospitalization endpoints in patients with heart failure when compared with an ACE inhibitor alone, although exercise duration (the primary endpoint) was not significantly better.15

Perspectives
The results of the present study in normal volunteers demonstrate that concomitant ACE and NEP inhibition has very favorable renal hemodynamic and tubular effects, as it reduces intraglomerular pressure and promotes sodium excretion. The present findings also provide clear evidence that NEP inhibition produces a sustained increase in urinary sodium excretion, which over 1 week can be quantitatively as important as the one induced by a thiazide diuretic. Thus, the principle of combining a NEP inhibitor with any blocker of the renin-angiotensin system, including an angiotensin receptor antagonist, holds particular promise for optimal management of hypertensive patients and possibly for slowing the progression of chronic renal failure. For the same reasons, such double blockade can be expected to exert favorable effects in patients with congestive heart failure.

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