Enhanced Natriuretic Response to Neutral Endopeptidase Inhibition in Heart-Transplant Recipients

Bernard Geny, Hélène Hardy, Jean Lonsdorfer, Bernard Eisenmann, Pascal Haberey, François Piquard

Abstract—Heart-transplant recipients (Htx) generally present with body fluid and sodium handling abnormalities and hypertension. To investigate whether neutral endopeptidase inhibition (NEP-I) increases endogenous atrial natriuretic peptide (ANP) and enhances natriuresis and diuresis after heart transplantation, ecadotril was given orally to 8 control subjects and 8 matched Htx, and levels of volume-regulating hormones and renal water, electrolyte, and cyclic guanosine monophosphate (cGMP) excretions were monitored for 210 minutes. Baseline plasma ANP, brain natriuretic peptide (BNP), and cGMP were elevated in Htx, but renin and aldosterone, like urinary parameters, did not differ between groups. NEP-I increased plasma ANP (Htx, 20.6±2.3 to 33.2±5.9 pmol/L, P<0.01; controls, 7.7±1.2 to 10.6±2.6 pmol/L) and cGMP, but not BNP. Renin decreased similarly in both groups, whereas aldosterone decreased significantly only in Htx. Enhanced urinary sodium (1650±370% versus 450±150%, P=0.01), cGMP, and water excretions were observed in Htx and urinary cGMP positively correlated with natriuresis in 6 of the Htx subjects. Consistent with a normal circadian rhythm of blood pressure, without excluding a possible effect of NEP-I, mean systemic blood pressure increased similarly in both groups at the end of the study (6.9±2.0% versus 7.4±2.8% in controls and Htx). Thus, systemic hypertension, mild renal impairment, and raised plasma ANP levels are possible contributory factors in the enhanced natriuresis and diuresis with NEP-I in Htx. These results support a physiological role for the cardiac hormone after heart transplantation and suggest that long-term studies may be useful to determine the potential of NEP-I in the treatment of sodium retention and water retention after heart transplantation. (Hypertension. 1999;33:969-974.)

Key Words: natriuresis, atrial natriuretic peptide, hormones, cardiac transplant, cardiac hypertension

Heart transplantation, a recognized treatment for end-stage congestive heart failure, improves significantly both the duration and quality of life of numerous patients. However, nearly all heart-transplant recipients (Htx) present with systemic hypertension. This complication appears to be unrelated to preexisting hypertension, and several factors including cardiac denervation and cyclosporine therapy are likely to participate in such blood pressure (BP) increase. Specifically, this hypertension is characterized by body fluid and sodium handling abnormalities and Htx may exhibit expansion of extracellular fluid volume and blunted or delayed renal response to volume expansion or redistribution.1-5

Atrial natriuretic peptide (ANP) has potent diuretic, natriuretic, and vasorelaxant properties and, in contrast to diuretic therapies, decreases the renin-angiotensin-aldosterone system (RAAS) activity.6,7 Abnormal BP and body fluid regulation in Htx, despite increased plasma ANP levels, has not ruled out a role for ANP in circulatory homeostasis after heart transplantation, since pharmacological doses of infused ANP have been shown to enhance natriuresis and diuresis in cyclosporine-treated Htx.8,9 In light of this, inhibition of the enzymatic degradation of the cardiac hormone by neutral endopeptidase 24.11 (NEP) may be a promising alternative to ANP intravenous infusion.10-12 Indeed, NEP is an important clearance pathway for ANP, and studies performed on animals and humans have demonstrated that neutral endopeptidase inhibition (NEP-I) enhances and prolongs the natriuretic effects of coinfused exogenous ANP and increases sodium excretion through an increase in endogenous ANP.13-16 Interestingly, greater natriuresis is observed when ANP levels are elevated, further supporting the potential role of NEP-I in Htx.17-20

Therefore, the aim of this study was to investigate for the first time whether NEP-I further increases circulating ANP after heart transplantation, and whether such increase is associated with enhanced diuresis and natriuresis in Htx as compared with control subjects.

Methods

Subjects

Eight healthy and 8 matched heart-transplant subjects, in sinus rhythm and cardiac symptom free, gave their informed consent and
participated in this study which was approved by the University Review Board for Human studies. Htx, free of rejection, received prednisolone (9.7±1.0 mg/d), cyclosporine (126±10 mg/d) and azathioprine (42.5±11.0 mg/d). Their antihypertension treatment (n=4), calcium antagonists (n=2), angiotensin conversion inhibitors (n=1), and/or furosemide (n=2), was withdrawn 8 days before the study. One control subject received low dose nitrates and a calcium antagonist for stable angina.

Study Design
Urine was collected from all subjects during the 24 hours before the experiment. No medication was given to Htx the morning of the study. All subjects received a light meal with hydration of 300 mL. After an initial 60-minute baseline period in the seated position, patients stood, voided, and then assumed a supine position for the remainder of the study. After a second 60-minute baseline period, the NEP-I (ecadotril 100 mg) was given orally. The follow-up period lasted 210 minutes. Nine venous blood samples were obtained through a 20 gauge intravenous catheter inserted into an antecubital vein. Subjects were asked to void freely at the end of each 60 minute baseline period and 60, 150, and 210 minutes after NEP-I. Systemic BPs, measured by the oscillographic method (Hewlett Packard PB S4S), and heart rate were recorded at 15 minute intervals.

Plasma and Urine Measurements
Urine and venous blood samples were handled on ice and hormone measurements were determined by radioimmunoassay with the use of commercially available kits as previously reported.3,21

Statistical Analysis
Results are expressed as mean±SEM. Comparisons were performed by use of 1-way or 2-way ANOVA, taking into account the effect of heart transplantation and NEP-I when needed, followed by Tukey’s test as needed to evaluate whether means of control and Htx were significantly different from baseline and from each other. Association between 2 groups of variables was assessed by calculating Pearson correlation coefficient in each subject on the values obtained before and after NEP-I. Statistical significance required a P<0.05.

Results
Baseline
The 2 groups were matched for age (47.6±3.6 versus 48.3±3.3 years) and body weight (77.9±4.3 versus 76.2±5.1 kg) in controls and Htx respectively. The average time since transplantation was 31.4±6.1 months. Htx had higher resting heart rate (84±5 versus 70±4 bpm, P<0.05) and systemic BP than controls (130±4 versus 120±6 mm Hg; 75±4 versus 63±3 mm Hg, P<0.05 and 94±4 versus 83±3 mm Hg, P<0.05, for systolic, diastolic, and mean BP, respectively). The 24 hour creatinine clearance was lower in Htx than in controls (80±6 versus 124±7 ml/min, P<0.01) but the 24 hour natriuresis, blood glucose, sodium, potassium, osmolality, and protein concentrations were similar in both groups.

Plasma ANP and BNP were significantly higher in Htx than in control subjects (20.7±3.1 versus 7.9±1.2 pmol/L, P<0.01 for ANP and 16.6±2.3 versus 7.1±0.8 pmol/L, P<0.01 for BNP). Similarly, plasma cGMP was significantly higher in Htx than in control subjects (8.8±1.0 versus 3.5±0.5 nmol/L, P<0.001). Plasma renin and aldosterone levels were not different between groups (346±54 versus 227±48 fmol/L for renin, 299±66 versus 229±52 pmol/L for aldosterone, in Htx and controls, respectively).

Effects of Neutral Endopeptidase Inhibition
Figure 1 outlines the hormonal response to NEP-I in controls and Htx. At all times, plasma ANP and cGMP concentrations were significantly higher in Htx than in controls. After administration of ecadotril, ANP increased markedly in Htx (from 20.6±2.3 to 33.2±5.9 pmol/L, P<0.01), the smaller increase observed in controls failing slightly to reach statistical significance (from 7.7±1.2 to 10.6±2.6 pmol/L). The time course of plasma cGMP paralleled that of ANP, both in controls and in Htx. Thus cGMP increased in Htx from 1.0±1.2 to 10.6±2.6 pmol/L. Positive linear correlation was observed between plasma ANP and cGMP values in most of the controls and Htx (Table 1). Circulating BNP remained higher in Htx after ecadotril intake (18.6±1.8 versus 7.6±1.0 pmol/L, P<0.001, in Htx and controls, respectively), but was not significantly modified in either group as compared with baseline values.

Plasma renin decreased similarly in both groups in response to NEP-I (from 227±48 to 128±22 fmol/L, P<0.01, in controls and from 346±54 to 235±27 pmol/L, P<0.01, in Htx). Plasma aldosterone decreased significantly in Htx (from 299±66 to 183±28 pmol/L, P<0.05) but not in controls (from 229±52 to 178±42 pmol/L).
Figure 2 shows the renal response to NEP-I in both groups. Sodium excretion increased from 64 ± 11 to 127 ± 76 μmol/min in controls, and from 38 ± 9 to 77 ± 35 μmol/min in Htx when position was changed from the seated to the supine position. It further increased after NEP-I to 272 ± 48 μmol/min, *P*, 0.05, in controls and to 395 ± 111 μmol/min, *P*, 0.01, in Htx. Cyclic GMP urinary excretion increased in controls from 0.41 ± 0.06 to 0.83 ± 0.12 μmol/min and in Htx from 0.83 ± 0.12 to 1.91 ± 0.29 μmol/min, *P*, 0.01. Significant positive correlations were observed between sodium and cGMP urinary excretions in most controls and Htx (Table 1).

Finally, water excretion increased from 0.7 ± 0.1 to 3.5 ± 0.6 mL/min, in controls, and from 0.8 ± 0.3 to 5.6 ± 1.5 mL/min, *P*, 0.01, in Htx. Although creatinine clearance tended to increase in Htx after ecadotril, this elevation failed to reach statistical significance and NEP-I did not modify controls’ creatinine clearance. Sodium reabsorption decreased significantly in both groups (from 99.6 ± 0.1% to 97.4 ± 0.4%, *P*, 0.01, in Htx, and from 99.7 ± 0.1% to 97.5 ± 0.4%, *P*, 0.01, in controls), whereas water reabsorption decreased significantly only in Htx (from 99.0 ± 0.4% to 95.6 ± 1.0%, *P*, 0.01).

Figure 3 shows the systemic BP time course before and after NEP-I. Systolic BP tended to increase only at the end of the study. Diastolic and mean BP did not change during the first 2 hours after NEP-I, then it increased slightly but significantly in both groups (6.9 ± 2.0% versus 7.4 ± 2.8% in controls and Htx, respectively). Heart rate remained significantly higher in Htx than in controls (P < 0.05 at all times), but a similar decrease was observed in both groups (from 69 ± 3 to 61 ± 4 bpm, P < 0.01 for controls and from 84 ± 4 to 77 ± 4 bpm, P < 0.05 for Htx.

Discussion

The main result of this study was to demonstrate that NEP-I further increases plasma ANP in Htx, thus enhancing natriuresis, diuresis, and urinary cGMP excretions as compared with control subjects. These data support ANP’s likely participation in body fluid homeostasis maintenance after cardiac transplantation and suggest that NEP-I may be a useful therapeutic tool in Htx.

Hormonal Effects of NEP-I in Htx

NEP-I increased circulating ANP both in Htx and controls. However, enzymatic and cellular pathways cooperate to degrade ANP in the body7 and thus, the receptor-mediated clearance pathway can buffer the effect of small increases in circulating ANP levels. This may explain why ANP increase did not reach statistical significance in controls. In Htx, the earlier and higher ANP increase suggests that the contribution of NEP to ANP degradation remains important. Thus, C-receptors, whose number has been shown to be reduced in patients with high ANP levels,22 have reduced capacity to buffer the effect of NEP-I when plasma ANP levels are elevated to the pathological range.23

Relationships Between Plasma ANP and cGMP and Between Natriuresis and cGMP Excretion in Controls and Htx

<table>
<thead>
<tr>
<th>Subject</th>
<th>Plasma ANP vs Plasma cGMP</th>
<th>UVNa vs UVcGMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl 1</td>
<td>0.85 0.004</td>
<td>0.98 0.02</td>
</tr>
<tr>
<td>Ctrl 2</td>
<td>0.76 0.02</td>
<td>0.85 0.07</td>
</tr>
<tr>
<td>Ctrl 3</td>
<td>0.64 0.06</td>
<td>0.95 0.10</td>
</tr>
<tr>
<td>Ctrl 4</td>
<td>0.66 0.06</td>
<td>0.91 0.20</td>
</tr>
<tr>
<td>Ctrl 5</td>
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</tr>
<tr>
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<td>0.88 0.04</td>
</tr>
<tr>
<td>Ctrl 7</td>
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<td>0.95 0.02</td>
</tr>
<tr>
<td>Ctrl 8</td>
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</tr>
<tr>
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<td>0.88 0.002</td>
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</tr>
<tr>
<td>Htx 2</td>
<td>0.66 0.06</td>
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<tr>
<td>Htx 3</td>
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<td>0.68 0.10</td>
</tr>
<tr>
<td>Htx 4</td>
<td>0.90 0.001</td>
<td>0.99 0.001</td>
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<tr>
<td>Htx 5</td>
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</tr>
<tr>
<td>Htx 8</td>
<td>0.78 0.01</td>
<td>0.96 0.04</td>
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</tbody>
</table>

Ctrl indicates controls.
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Figure 3. Time course of systemic blood pressure (s, systolic; d, diastolic; m, mean) changes before and after NEP-I in Htx (●) and in controls (○). Difference with corresponding values before the administration of drug: §P<0.05, *P<0.01.

Controversial results have been reported concerning the effect of NEP-I on BNP. Thus, it has been demonstrated in vitro that BNP is an NEP substrate and potentiating of the biological effects of BNP has been observed in animals pretreated with NEP-I. Furthermore, candoxatril has increased plasma BNP in patients with heart failure. However, candoxatril has had little effect on circulating BNP in hypertensive patients and accordingly, BNP circulating levels failed to change after NEP-I in both our control subjects and Htx. This suggests that NEP does not play as pivotal a role in BNP clearance as it does in the clearance of ANP.

NEP-I has been previously reported to decrease the RAAS and, accordingly, the time course and the degree of renin decrease in our 2 groups of subjects are consistent with studies performed in animals, control subjects, and patients with severe heart failure. However, plasma aldosterone decreased significantly only in Htx. Because renin decreased similarly in both Htx and controls, it suggests that the greater aldosterone inhibition in Htx could also be caused by an ANP-induced direct inhibition of aldosterone production. Accordingly, endopeptidase 24.11 has been localized in the glomerulosa cells, where it may directly inactivate ANP. Consistently, intravenous candoxatril has been reported to decrease plasma aldosterone values without affecting circulating renin.

Renal Effects of NEP-I in Htx

NEP-I increased significantly the sodium, water, and urinary cGMP excretions in both groups, without a significant change in creatinine clearance. This is in accordance with previous studies with ecdotril or other NEP-inhibitors showing either no changes or a slight increase in glomerular filtration rate. Although moderate, the plasma ANP increase in control subjects was associated with increased natriuresis, further supporting the hypothesis that NEP-I might prevent local intrarenal degradation of ANP.

Interestingly, larger natriuretic response was observed in Htx. Increased BP, differences in sodium intake, mild renal failure, and/or exaggerated plasma ANP increase after NEP-I may explain such results. Because the increase in mean systemic BP was not different between controls and Htx and occurred 2 hours after NEP-I, that is, 1 hour after the maximal diuresis and natriuresis was seen in both groups, it is likely that a pressure-dependent mechanism could not explain totally the renal responses observed. Accordingly, NEP-I has been shown to increase natriuresis and diuresis in the absence of BP change. Dietary sodium intake also is an important determinant of the renal response to NEP-I, the sodium excretion being greater when subjects are on a high sodium diet.

As inferred from the 24 hours natriuresis, although not statistically different, the sodium intake was lower in Htx as compared with controls. Thus, the effect of sodium intake on the renal responses to NEP-I, if any, should have been a reduction in water and sodium renal excretions in Htx.

Enhanced natriuretic response to NEP-I has been recently reported in patients with moderate chronic renal failure. The authors proposed that the greater natriuretic response may reflect the high plasma ANP levels achieved in these uremic patients. This hypothesis may also apply to Htx and, thus, their mild renal failure could contribute to their increased renal response to NEP-I through their greater baseline and NEP-I induced ANP elevation. Consistently, ANP is known to enhance water and sodium excretions through a decrease in tubular reabsorption. The concomitant plasma ANP and cGMP increases and the positive correlation between urinary cGMP excretion and natriuresis observed in a majority of Htx also strongly support the participation of ANP in the water and sodium excretions handling after NEP-I in Htx. Indeed, despite the fact that NEP is involved in the degradation of a number of peptides that may affect natriuresis, urinary cGMP has been shown to be a good marker of the renal activity of ANP. Accordingly, anti-ANP antiserum has been shown to prevent the natriuretic effect and the increase in urinary cGMP induced by NEP-I. Thus, although this study did not deny that mechanisms other than ANP may be involved, our data support a role for endogenous ANP in the enhanced renal response observed in Htx after NEP-I. These results may be particularly interesting in Htx, in view of their systemic hypertension generally associated with salt and water retention.

Potential Advantages or Disadvantages of NEP-I

Versus Conventional Diuretic Agents

Hypertension, very common in Htx, is difficult to treat, and many patients require more than one antihypertensive agent.
Thus, conventional diuretics are essential for the management of volume expansion in Htx, despite their favoring action on diabetes, gout, and cyclosporine nephrotoxicity.2,36 In view of this, NEP-I may be interesting since ANP counteracts the antinatriuretic, preglomerular vasoconstrictor, hypertensive, mesangial constrictor, and sympathetic neural stimulatory effects of cyclosporine.6–9,36 Furthermore, the magnitude of the urinary response to ecadotril seems to be related to the volume status of the subject.18,32 Conventional diuretics also generally activate the RAAS and may reduce plasma ANP, thus impairing a compensatory mechanism resisting sodium retention. The ability of NEP-I to reduce the RAAS activity is an exceptional property for a diuretic and represents another reason in favor of such a treatment.9,20,30

Study Limitations

Normal subjects can be considered as controls, when comparing NEP-I in Htx and normal subjects, but a placebo study could have been useful considering the effects of the supine position and the increase in BP observed in each group. However, according to the literature, we designed a relatively long baseline period in the supine position so that the hormonal and renal parameters determined should have been stable. Thus, although moving from the seated to supine position may decrease plasma renin and aldosterone, increase ANP and natriuresis, such physiological responses are generally obtained within 1 hour. Indeed, the RAAS responds quickly to a postural change and maximal ANP increase occurs 45 to 60 minutes after atrial distention. Accordingly, the 2 baseline plasma ANP values were similar, both in Htx and controls. Similarly, increased natriuresis in the supine position occurs mainly during the first hour after a positional change, natriuresis remaining unmodified thereafter. Furthermore, as we observed, such positional change generally increases the natriuresis by about 100%, to be compared with the further 400% natriuresis increase observed after NEP-I in Htx.3,37–39 Thus, both the magnitude and the time course of natriuresis after moving to the supine position, in view of the length of our baseline period, support that the further natriuresis increase observed after NEP-I is because of the drug activity. Several studies demonstrated that NEP-I, including ecadotril, has either no effect or decreases BP in normal subjects and in heart failure, renal failure, cirrhosis, and hypertensive patients.9 Furthermore, in contrast with the enhanced hormonal and renal actions of NEP-I in Htx, BP increased similarly in Htx and controls. Although these data cannot eliminate a possible ecadotril-induced increase in BP, they are consistent with a normal circadian rhythm of BP both in controls and Htx, and may suggest that the physiological BP morning-daytime acrophase reappearing late after cardiac transplantation explains such small BP increase.42

In conclusion, this study demonstrates an NEP-I–induced enhanced natriuresis and diuresis in Htx as compared with control subjects. Plasma ANP increase is likely to participate in such exaggerated renal responses, suggesting a beneficial role of the cardiac hormone. These results support the assumption that NEP inhibitors may play a therapeutic role in the treatment of sodium and water retention after heart transplantation and that a long-term study may be justified to evaluate the usefulness of NEP inhibitors for the treatment of hypertension in Htx.

Acknowledgments

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References

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