Hormonal, renal, hemodynamic responses to acute neutral endopeptidase inhibition in heart transplant patients

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Atrial natriuretic peptide (ANP) is a potent vasodilatory, diuretic, and natriuretic hormone, mainly of cardiac origin, that is suggested to protect the body against volume and/or pressure overloads both in normal humans and in patients with cardiovascular disease (4, 6). Nevertheless, heart transplant patients (HTPs), despite their increased circulating cardiac natriuretic peptides, often present with systemic hypertension and volume overload associated with mildly impaired renal function. This may suggest a relative renal hyporesponsiveness to ANP, supporting the need to further increase the cardiac hormone after heart transplantation (16, 19, 36).

On the basis of these findings, neutral endopeptidase (NEP) inhibition, the main enzyme that catalyzes the degradation of ANP, could be an attractive therapeutic approach (9, 10, 22, 30, 34). Indeed, NEP inhibition increases circulating ANP, and several reports demonstrated that the greater the baseline ANP level the greater the renal response to NEP inhibition (12, 13, 28). Furthermore, acute reversal of cyclosporine nephrotoxicity by NEP inhibition has been reported in stable renal transplant recipients (29).

Nevertheless, studies also raised the issue of a possible deleterious effect of NEP inhibition on circulatory homeostasis (2, 14, 17, 23). Thus recent data showed that NEP inhibition can increase systemic vascular resistance during congestive heart failure (23) or cause vasoconstriction of human resistance vessels in vivo (14). The latter may be mediated at least partly by endothelin (ET)-1, because concomitant ETA-receptor blockade inhibited NEP inhibition-induced vasoconstriction (14). Accordingly, increased blood pressure, together with increased circulating ET-1, has been reported after NEP inhibition but not after ANP infusion in healthy men (2). Similarly, our laboratory recently observed an increased blood pressure in both controls and HTPs in response to NEP inhibition. However, a placebo group was not included in the study, and circulating ET-like precise renal hemodynamics were not investigated (17).

The aim of the present study was, therefore, to determine whether a NEP inhibition-induced increase in ET could be responsible for the systemic hypertension previously reported in HTPs. We additionally investigated whether increased endogenous ANP could participate in natriuresis enhancement after heart transplantation, through a glomerular and/or a tubular mechanism.
MATERIALS AND METHODS

Subjects. Seven chronic (44 ± 9 mo after transplantation) HTPs who were otherwise in good health participated in the study. They signed a written consent form, and the study was approved by the University Review Board for Human Studies. All subjects (47 ± 12 yr old and 69 ± 7 kg) were men in sinus rhythm and were cardiac symptom and rejection free, as inferred from the later right ventricular biopsy. They were under the usual triple-drug immunosuppressive therapy including prednisolone (6 ± 2 mg/day), azathioprine (50 ± 5 mg/day), and cyclosporine (143 ± 10 mg/day). Four subjects were under antihypertensive therapy such as calcium antagonists (n = 2) and nitrates (n = 2). The therapy had been maintained by each patient for at least 3 mo.

Study design. Two double-blind experiments were performed at 8-day intervals, with each subject serving as his own control and randomly receiving either placebo or the oral NEP inhibitor [ecadotril (200 mg), Laboratoire Bioprojet, Paris, France]. In the appropriate subjects, antihypertensive regimen was withdrawn 8 days preceding the study; the sodium and water content of their diets was similar to those voided (except for urinary data). Healthy volunteers were not included in this study because comparison of their specific response to NEP inhibition with that of HTPs has been previously reported (17). Nevertheless, we present resting response to NEP inhibition with that of HTPs has been included in this study because comparison of their specific response to NEP inhibition with that of HTPs has been previously reported (17). Nevertheless, we present resting characteristics of 11 age- and weight-matched healthy controls to allow comparisons with HTPs.

The equilibrium period lasted 60 min during which, after a priming, a constant-rate infusion (90 ml/h) of inulin (Inutest, Nephrotest, Biologische Arbeitsgemeinschaft, Lich, Germany) and then the subjects freely voided every 60 min later by oral ecadotril (200 mg) or placebo. The follow-up period lasted 360 min to ensure recording of possible late renal hemodynamic modification. Hemodynamic measurements (every 30 min) followed by blood withdrawal from an intravenous cannula (18 gauge) were obtained while the subjects were in the supine position, and then the subjects freely voided every 60 min.

Variables determined. Systemic arterial pressures were monitored with an automatic noninvasive oscillometric technique. Plasma and urine electrolytes, hematocrit, plasma ANP and brain natriuretic peptide (BNP), ET-1, and cGMP were determined by specific electrodes or radioimmunoassays, as previously reported (8). Plasma and urine inulin concentrations were determined according to an enzymatic method as previously described (8). Plasma and urine PAH concentrations were determined by colorimetric method adapted on a Technicon AutoAnalyzer (Technicon Instrument, Tarrytown, NY) (34). Plasma and urine inulin concentrations were determined according to an enzymatic method as previously described (8). Plasma and urine PAH concentrations were determined by colorimetric method adapted on a Technicon AutoAnalyzer (Technicon Instrument, Tarrytown, NY) (34).

Calculations. Clearances (C) were calculated according to the standard formula \[C = \frac{U \times V}{P}\], where, for a given substance, U is the urine concentration, V is the urinary flow, and P is the plasma concentration. Inulin and PAH clearances were taken as glomerular filtration rate (GFR) and renal plasma flow (RPF), respectively. Filtration fraction was calculated as GFR/RPF. Renal vascular resistance (RVR; in kdyn·cm⁻²·s) was calculated as \[(MAP/RBF) \times 80/1,000\], where RBF is renal blood flow and with mean arterial pressure (MAP) being calculated as the average of all MAPs recorded during the clearance procedure.

Statistical analysis. Results are expressed as means ± SE. Comparisons between two means were assessed by one-way analysis of variance. Multiple comparisons were performed by using two-way analysis of variance with repeated measures, taking into account the effect of drug (NEP inhibitor or placebo) and time after administration when needed. Tukey’s test was used, when analysis of variance was significant, to evaluate whether means of placebo and NEP inhibition were significantly different from baselines and from each other. Relationships between two groups of variables were assessed by calculating Pearson correlation coefficient in each subject for the values obtained before and after NEP inhibition or placebo. Statistical significance required a value of \(P < 0.05\).

RESULTS

Baseline data. The baseline hemodynamic, renal, and hormonal characteristics of the subjects are shown on Table 1. All parameters, including 24-h natriuresis, diuresis, plasma, and urinary electrolytes, were similar before placebo or NEP inhibition. HTPs presented with a moderate increase in systemic blood pressure and heart rate, associated with a decreased glomerular filtration rate compared with normal values obtained in a group of 11 age- and weight-matched healthy subjects (124 ± 5 mmHg for systemic systolic pressure, 67 ± 4 mmHg for diastolic pressure, 87 ± 4 mmHg for mean blood pressure, 71 ± 3 beats/min for heart rate, and 142 ± 17 ml/min for creatinine clearance). Similarly, circulating ANP, BNP, and cGMP were increased in HTPs compared with normal values (7.0 ± 0.8 and 7.6 ± 0.6 pmol/l \((P < 0.001)\) for ANP and BNP, respectively, and 3.8 ± 0.5 nmol/l \((P < 0.01)\) for cGMP).

Table 1. Baseline hemodynamic, renal, and hormonal characteristics of the subject groups before placebo or NEP inhibition

<table>
<thead>
<tr>
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<th>Placebo</th>
<th>Ecadotril</th>
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<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>89 ± 3</td>
<td>85 ± 4</td>
</tr>
<tr>
<td>Systolic SBP, mmHg</td>
<td>146 ± 8</td>
<td>147 ± 8</td>
</tr>
<tr>
<td>Diastolic SBP, mmHg</td>
<td>85 ± 4</td>
<td>85 ± 5</td>
</tr>
<tr>
<td>Mean SBP, mmHg</td>
<td>165 ± 4</td>
<td>107 ± 6</td>
</tr>
<tr>
<td>24-h natriuresis, mmol/24 h</td>
<td>101 ± 21</td>
<td>114 ± 20</td>
</tr>
<tr>
<td>24-h diuresis, l/24 h</td>
<td>2.5 ± 0.5</td>
<td>2.7 ± 0.5</td>
</tr>
<tr>
<td>Diuresis, ml/min</td>
<td>5.0 ± 1.0</td>
<td>4.9 ± 1.0</td>
</tr>
<tr>
<td>Natriuresis, µmol/min</td>
<td>148 ± 35</td>
<td>159 ± 40</td>
</tr>
<tr>
<td>cGMP (excreted), nmol/min</td>
<td>1.04 ± 0.17</td>
<td>0.99 ± 0.21</td>
</tr>
<tr>
<td>C_inu, ml/min</td>
<td>86 ± 4</td>
<td>78 ± 6</td>
</tr>
<tr>
<td>C_PAH, ml/min</td>
<td>383 ± 45</td>
<td>376 ± 39</td>
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<tr>
<td>Creatinine, µmol/l</td>
<td>23.5 ± 0.02</td>
<td>21.7 ± 0.02</td>
</tr>
<tr>
<td>Plasma ANP, pmol/l</td>
<td>22.6 ± 2.4</td>
<td>21.5 ± 2.7</td>
</tr>
<tr>
<td>Plasma BNP, pmol/l</td>
<td>27.8 ± 3.5</td>
<td>30.9 ± 4.9</td>
</tr>
<tr>
<td>Plasma cGMP, nmol/l</td>
<td>9.2 ± 1.5</td>
<td>7.8 ± 2.7</td>
</tr>
<tr>
<td>Plasma endothelin-1, pmol/l</td>
<td>2.3 ± 0.2</td>
<td>2.1 ± 0.2</td>
</tr>
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</table>

Values are means ± SE. NEP, neutral endopeptidase; SBP, systemic blood pressure; C_inu, inulin clearance; C_PAH, p-aminohippuric acid clearance; FF, filtration fraction; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide.
Effects of neutral endopeptidase inhibition. As expected, placebo administration failed to significantly modify any hormonal, renal, or hemodynamic parameters.

Figure 1 illustrates the hormonal responses to NEP inhibition, compared with placebo. NEP inhibition significantly increased plasma ANP (from 21.5 ± 2.7 to 29.6 ± 3.7 pmol/l; P < 0.01), cGMP (from 7.8 ± 2.7 to 14.0 ± 4 nmol/l; P < 0.01), and endothelin-1 (from 2.01 ± 0.1 to 2.90 ± 0.2 pmol/l; P < 0.01). When the relative change from baseline to peak of each HTP was averaged, ET-1 increased by 50.8 ± 12.1% (P < 0.001) after NEP inhibition and by 5.6 ± 5.0% (P = not significant) after placebo, with such increase after NEP inhibition being significant compared with placebo values (P = 0.005). Circulating BNP failed to change after ecadotril administration (from 30.9 ± 4.9 to 32.5 ± 3.6 pmol/l).

Figure 2 shows blood cyclosporine concentrations, inulin and PAH clearances, and filtration fraction time courses. Cyclosporine blood levels increased sharply after cyclosporine administration and decreased thereafter progressively. Renal plasma flow and glomerular filtration rate were not significantly modified after NEP inhibition and failed to differ between placebo and ecadotril groups. However, filtration fraction significantly increased after NEP inhibition (from 21.7 ± 2.5 to 27.6 ± 1.8% (P < 0.05) for a mean increase of 33 ± 13%).

Figure 2 shows blood cyclosporine concentrations, inulin and PAH clearances, and filtration fraction time courses. Cyclosporine blood levels increased sharply after cyclosporine administration and decreased thereafter progressively. Renal plasma flow and glomerular filtration rate were not significantly modified after NEP inhibition and failed to differ between placebo and ecadotril groups. However, filtration fraction significantly increased after NEP inhibition (from 21.7 ± 2.5 to 27.6 ± 1.8% (P < 0.05) for a mean increase of 33 ± 13%).

Figure 3 shows natriuresis, sodium reabsorption, and cGMP urinary excretion in both arms of the experiment. Outlining the lack of effect of placebo, it presents an enhanced renal response after NEP inhibition. Thus sodium excretion increased from 159 ± 40 to 651 ± 63 µmol/min (P < 0.01), corresponding to a mean increase of 315 ± 105%. Sodium-filtered load failed to increase significantly, but sodium reabsorption decreased sharply after NEP inhibition. The time course of urinary cGMP paralleled that of natriuresis after
NEP inhibition, increasing from 0.95 ± 0.22 to 2.10 ± 0.40 nmol/min (P < 0.01). Similarly, urine output increased by 196 ± 62% from 4.9 ± 1.0 to 9.3 ± 0.7 ml/min (P < 0.01) only after ecadotril.

Figure 4 illustrates mean systemic blood pressure time course in both groups. Despite some fluctuations, mean systemic blood pressure failed to change significantly after either placebo or NEP inhibition. Similarly, systolic and diastolic blood pressures remained unmodiﬁed.

Interestingly, total renal resistance was signiﬁcantly increased 120 min after drug administration in the placebo group (from 13.8 ± 1.1 to 20.3 ± 3.1 kdyn·cm⁻⁵·s⁻¹; P < 0.05) but failed to change signiﬁcantly in the ecadotril group (from 15.0 ± 2.3 to 18.4 ± 1.7 kdyn·cm⁻⁵·s⁻¹).

Table 2 presents the relationships among plasma ANP and cGMP, natriuresis, and sodium reabsorption. Increased correlation coefﬁcient, together with higher statistical signiﬁcance, was a general ﬁnding after NEP inhibition, compared with placebo. ANP correlated positively with both plasma and urinary cGMP and sodium excretion. Interestingly, however, stronger correlations were observed with urinary cGMP. Thus urinary cGMP and sodium excretion were positively related on one hand, urinary cGMP and sodium reabsorption being negatively related (r = −0.71, P < 0.0001) on the other hand, after NEP inhibition.

DISCUSSION

The main results of this study are to demonstrate that, despite increasing ET-1, NEP inhibition did not adversely affect HTPs’ systemic hemodynamic and renal function. Furthermore, NEP inhibition reduces the increase in renal vascular resistance seen after placebo (cyclosporine alone) and induces an ANP elevation associated with increased cGMP (plasma and urinary), increased natriuresis, and decreased sodium reabsorption.

Cardiac natriuretic peptides, ET, and heart transplantation. After successful cardiac transplantation, despite normalization of ﬁlling pressures, both ANP and BNP remain generally elevated (16, 19, 36). Consistently, both cardiac hormones’ plasma levels are increased in our HTPs, compared with normal values. Several hypothesis have been raised to explain such ﬁndings, and both volume and/or pressure overload,

![Figure 3. Urinary excretion of sodium (A), fractional sodium reabsorption (B), and cGMP excretion (C) before and after neutral endopeptidase inhibition (ecadotril) or placebo in heart transplant recipients (arrow, time of administration).](image)

![Figure 4. Time course of mean systemic blood pressure (SBP) before and after neutral endopeptidase inhibition (ecadotril, ●) or placebo (○) in heart transplant patients (arrow, time of administration).](image)

Table 2. Relationships between plasma ANP, cGMP, natriuresis, and sodium reabsorption in subjects receiving placebo or ecadotril

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<th>Ecadotril</th>
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<tr>
<td></td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>ANP vs. ANP</td>
<td>0.32</td>
<td>0.06</td>
</tr>
<tr>
<td>cGMP (plasma)</td>
<td>0.36</td>
<td>0.03</td>
</tr>
<tr>
<td>cGMP (excreted)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natriuresis vs. ANP</td>
<td>0.25</td>
<td>0.117</td>
</tr>
<tr>
<td>cGMP (plasma)</td>
<td>−0.12</td>
<td>0.49</td>
</tr>
<tr>
<td>cGMP (excreted)</td>
<td>0.33</td>
<td>0.02</td>
</tr>
<tr>
<td>Sodium reabsorption vs. ANP</td>
<td>0.16</td>
<td>0.38</td>
</tr>
<tr>
<td>cGMP (plasma)</td>
<td>0.005</td>
<td>0.97</td>
</tr>
<tr>
<td>cGMP (excreted)</td>
<td>−0.29</td>
<td>0.04</td>
</tr>
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</table>

r, Pearson correlation coefficient.
together with diastolic dysfunction, should play a key role in the cardiac natriuretic system stimulation observed after heart transplantation (15–17, 19, 21, 36). Similarly, circulating ET is often increased after heart transplantation. This likely results from decreased metabolism and increased release secondary to renal and vascular dysfunctions, which are enhanced by the cyclosporine therapy (18).

**ET, hemodynamics, and neutral endopeptidase inhibition after cardiac transplantation.** Previous data demonstrated that NEP played a role in the inactivation of ET, modulating its circulating concentration (1, 31). Accordingly, NEP inhibition increases slightly but significantly and durably circulating ET after heart transplantation, suggesting that harmful effects of ET, such as vasoconstriction and decline in renal function, could be expected. Indeed, although ET<sub>R</sub>-receptor stimulation may trigger natriuresis, reduced vasodilatory response to low-dose ET has been observed after heart transplantation (5). Furthermore, increased ET has generally been shown to result in systemic hypertension and decreased renal plasma flow, glomerular filtration rate, and natriuresis (1, 18, 35).

Interestingly, however, NEP inhibition did not result in systemic hypertension in our HTPs. This finding is in opposition to a previous report showing a significant blood pressure increase in HTPs after NEP inhibition with 100 mg ecadotril (17). The clinical characteristics of the subjects being similar, such discrepancy may be explained by methodological differences (drug dosage and/or study design). A higher dose of ecadotril was given in the present study (200 mg) to obtain a longer duration of the NEP-induced ANP increase. In fact, both duration and amplitude of the cardiac hormone increase are comparable to those observed after 100 mg ecadotril, supporting similar NEP inhibitor biological activity of both dosages. Therefore, such blood pressure response discrepancy could not be attributed to differences in dosage. Concerning the design, this study began also in the morning, and, noteworthy, we still observed a blood pressure morning-daytime acrophase similar to that previously reported (17). However, this study lasted longer, and thus later slight blood pressure changes could explain why the late-morning systemic blood pressure increase failed to reach statistical significance. Furthermore, because HTPs presented a similar blood pressure pattern after placebo, such increase likely corresponds to blood pressure variations rather than to a direct effect of NEP inhibition. Accordingly, reappearance of a normal circadian rhythm of blood pressure has been reported after cardiac transplantation (21).

Concerning the renal function, the lack of NEP inhibition-induced adverse effects is particularly relevant after heart transplantation. Indeed, similar ET plasma levels caused vasoconstriction of human resistance vessels (14), and the particular sensitivity of renal resistance vasculature to ET-1 was enhanced in subjects under cyclosporine therapy. Although increased ET-1 plasma levels could explain why renal plasma flow and glomerular filtration rate fail to increase after NEP inhibition (increases being expected in view of the glomerular actions of ANP), ET-1 elevation does not result in further renal vasoconstriction (35). Thus, contrasting with the cyclosporine-induced daily renal hypoperfusion observed in renal transplant recipients, renal plasma flow remains unchanged after heart transplantation (29).

Although other mechanisms may be involved (implicating known or other yet unknown vasoactive or natriuretic peptides), because ANP inhibits ET production and actions (24, 38), one explanation for the lack of ET-mediated global deleterious effect after NEP inhibition might be the molar ratio of ET to ANP. Indeed, Ota et al. (32) demonstrated that only high ET concentrations (i.e., one-half that of ANP on the molar ratio) completely counteract the vascular, hormonal, and renal effects of ANP. In our study, although NEP inhibition increases significantly ET, the molar ratio of ET to ANP remains low. Both the elevated baseline cardiac hormone level and the further NEP inhibition-induced ANP increase could account for such an effect.

Alternatively, recent data demonstrated that ET<sub>A</sub> receptors can be downregulated in patients with cardiovascular disease (26). Thus downregulation of the ET-1 receptor could be an adaptive response to increased levels of ET-1 after heart transplantation, resulting in reduced biological effects from ET-1. Further reducing concerns that local vascular effects of NEP inhibition might be unfavorable, a recent in vitro study demonstrated that NEP inhibition does not augment the vasoconstrictor activity of ET in resistance arteries from patients with chronic heart failure (33).

**Pathophysiologial role of ANP after cardiac transplantation.** Although potentially useful in this issue, natriuretic peptide inhibition cannot be applied in humans. Nevertheless, in animals, administration of intrarenal natriuretic peptide receptor antagonist attenuates the renal properties of omapatrilat, a vaso-peptidase inhibitor (10). This suggests that, besides other peptides that might also be NEP substrates, ANP enhanced availability could be involved in the NEP inhibition-induced natriuresis observed in HTPs.

Indeed, there is substantial evidence to support such a hypothesis. In accordance with the well-known biological actions of ANP, NEP inhibition increased renal filtration fraction and dramatically decreased sodium tubular reabsorption (4, 6), together with an increase in plasma ANP, plasma cGMP, and urinary cGMP. Plasma cGMP is the second messenger of several peptides, but urinary cGMP is generally considered as a good marker of ANP renal activity (37, 39). Accordingly, although correlation does not imply causation, increased plasma ANP is strongly correlated with increased urinary cGMP, which in turn correlates positively with natriuresis and negatively with sodium reabsorption. Interestingly, however, plasma cGMP is only weakly related to natriuresis and inversely related to sodium tubular reabsorption. In view of the previously demonstrated appearance of ANP in urine, the correlations further suggest that NEP inhibition increases the natriuretic response to ANP, probably by...
potentiating the action of the filtrated peptide (20). Thus inhibition of NEP-induced anpeptidase degradation in the brush border membrane of the proximal tubules allows increased binding of filtered natriuretic peptide to ANP-receptor sites in the distal tubules, resulting in the ANP-potentiating natriuretic effect of the NEP inhibitor (11, 20).

Besides increasing natriuresis through a tubular mechanism, increased cardiac natriuretic peptide could also have a protective effect against acute cyclosporine nephrotoxicity after heart transplantation. Indeed, in contrast to our results, it is noteworthy that similar cyclosporine doses decrease both glomerular filtration rate and renal blood flow in renal transplant patients characterized by low ANP levels (28, 29). Thus, although renal resistance increases during the placebo arm of the study (cyclosporine alone), it is not associated with a decrease in renal blood flow in HTPs. Furthermore, the renal resistance increase observed after placebo is blunted after NEP inhibition. Consistently, exogenous ANP ameliorates the reduction in glomerular filtration rate induced by cyclosporine (27), and NEP inhibition has recently been shown to potentiate the effect of ANP on acute cyclosporine-induced nephrotoxicity (7).

Whether such a “protective” effect of NEP inhibition is mainly due to ANP or other peptides cannot be directly inferred from our data. However, a sympathoinhibitory effect of ANP, possibly counterbalancing a sympathoexcitatory action of cyclosporine, might be involved. Indeed, ANP has sympathoinhibitory effects in normal subjects and probably also in individuals with heart failure. Such an effect may act in concert with the natriuretic, diuretic, and vasodilator actions of endogenous ANP (3, 25). In our study, diastolic blood pressure remained unchanged, suggesting that sympathethetic activation secondary to arterial baroreceptor unloading was unlikely (3). Thus ANP sympathoinhibitory action might have been strong enough to participate in blood pressure and renal perfusion modulation in our subjects. This issue deserves further studies.

In conclusion, this study demonstrates that, despite increasing ET-1, NEP inhibition did not adversely affect systemic and renal hemodynamics after heart transplantation. Although other peptides might be involved, this integrated study further supports a pathophysiologica role for increased ANP, which is likely participation in the enhancement renal filtration fraction, diuresis, and natriuresis observed after NEP inhibition. Therapeutic strategies increasing natriuretic peptide concentration in HTPs may therefore be warranted.

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REFERENCES


