Plasma Urotensin in Human Systolic Heart Failure

Leong L. Ng, MD; Ian Loke, MB; Russell J. O’Brien, MB; Iain B. Squire, MD; Joan E. Davies, PhD

Background—Human urotensin II (UTN) has potent vasoactive and cardiostimulatory effects, acting on the G protein–linked receptor GPR14. Myocardial UTN expression is upregulated in heart failure (HF; left ventricular systolic dysfunction) in comparison with plasma N-terminal pro-brain natriuretic peptide (N-BNP) levels.

Methods and Results—N-BNP and UTN were measured in plasma from 126 patients with HF and 220 age- and sex-matched controls. Both peptides were elevated in plasma of HF patients and were correlated (r = 0.35, P < 0.001). In contrast to N-BNP, there was no relationship of plasma UTN with New York Heart Association (NYHA) class. Although plasma N-BNP showed a positive relationship with age and female sex, there was no such age-dependent change in plasma UTN, and control women had lower levels compared with control men. Receiver operating characteristic curves for the diagnosis of HF had areas of 0.90 and 0.86 for N-BNP and UTN, respectively (P < 0.001 for both). Receiver operating characteristic curve area for diagnosis of NYHA class I HF with UTN was better than that with N-BNP.

Conclusions—Plasma UTN is elevated in HF, which suggests a pathophysiological role for this peptide. Plasma UTN may be a useful alternative to N-BNP in the diagnosis of HF, inasmuch as its levels are elevated irrespective of age, sex, or NYHA class. (Circulation. 2002;106:2877-2880.)

Key Words: heart failure ■ diagnosis ■ natriuretic peptides

Heart failure (HF; left ventricular systolic dysfunction) is a leading cause of mortality and morbidity. Its pathophysiology involves activation of many neurohormonal systems. Recent work has revealed the existence of a novel cardiovascular peptide called urotensin II (UTN) with homology to the teleost fish hormone. This cyclic undecapeptide is the ligand for an orphan G protein receptor (GPR14), and both peptide and receptors are distributed within the cardiovascular and nervous systems. Although a potent vasoconstrictor for certain vascular beds in rats and monkeys, it may be vasodilator in mesenteric resistance vessels. There are important species differences in the reactivity of different vessels to UTN. For example, UTN vasoconstricts monkey vessels, vasodilates human pulmonary vasculature and mesenteric resistance vessels, and has no effect on human subcutaneous resistance vessels. UTN also causes vascular smooth muscle hypertrophy. Direct effects on the monkey myocardium include myocardial depression, although in human myocardium, a positive inotropic effect was demonstrated. In addition, hypertrophic effects and a possible role in ventricular remodeling have been described. UTN increased expression of the atrial and brain natriuretic peptides in cardiomyocytes, a finding expected in HF. Recent work documented increased UTN and GPR14 expression in myocardium of HF patients. We and others have documented increased plasma N-terminal brain natriuretic peptide (N-BNP) levels in HF. In view of the above cardiovascular actions of UTN and its stimulation of myocardial BNP synthesis, we investigated plasma UTN levels in human HF and compared them with levels of N-BNP.

Methods

Plasma Samples
Patients with HF were recruited from the hospital. All had echocardiographically confirmed ejection fractions <45%. Controls were age and sex matched and had ejection fractions >55% (Table). The local ethics committee approved the study. After 15 minutes’ bed rest, 10 mL blood was collected into tubes containing EDTA and aprotinin. Plasma was stored at −70°C until assayed.

N-BNP Assay
Our N-BNP assay was based on a noncompetitive assay. Rabbit antibodies were raised to the N- and C-terminals of human N-BNP. The C-terminal–directed IgG was the capture antibody. The N-terminal IgG was affinity-purified and biotinylated. Samples or N-BNP standards were incubated in C-terminal IgG–coated wells with the biotinylated antibody for 24 hours at 4°C. Detection was with methyl-acridinium ester (MAE)–labeled streptavidin. The lower limit of detection was 5.7 fmol/mL. Within- and between-assay coefficients of variation were 2.3% and 4.8%, respectively.
There was no crossreactivity with atrial natriuretic peptide, BNP, or C-type natriuretic peptide.

**Urotensin II Assay**
Antibody specific for UTN was obtained from Peninsular Laboratories, Calif. Biotinylated UTN purified on reverse-phase high-pressure liquid chromatography served as the tracer. A competitive assay with C18 extracts of plasma was utilized, incubating 50 ng of the antibody with extracts or standards in assay buffer (as described in reference 13). After 24 hours of incubation at 4°C, biotinylated UTN tracer was added (250 fmol/well). Immunoprecipitates were recovered in anti-rabbit IgG–coated ELISA plates. After washes and incubation with streptavidin-MAE, chemiluminescence was elicited as described.13 Intra- and interassay coefficients of variation were 2.3% and 8.1% respectively, with no reactivity for BNP or N-BNP. The lower limit of detection was 3.1 fmol/mL.

**Size-Exclusion Chromatography**
Plasma extracts were fractionated by isocratic size-exclusion chromatography on a Bio-Sep S2000 column (Phenomenex) with 50 mmol/L NaH₂PO₄ (pH 6.8) at a flow rate of 1 mL/min. Standards used to establish molecular weights included IgG, bovine albumin, ovalbumin, soybean trypsin inhibitor, aprotinin, and recombinant urotensin. Fractions collected every 30 seconds were dried before UTN assay.

**Statistical Analysis**
Statistical analyses were performed on SPSS Version 11.

**Results**
Fractionation of plasma extracts on size-exclusion chromatography produced one peak of immunoreactivity that eluted in the same position as recombinant human UTN (Figure 1A).

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Controls</th>
<th>Heart Failure Patients</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>220</td>
<td>126</td>
<td></td>
</tr>
<tr>
<td>Female sex, n (%)</td>
<td>78 (35)</td>
<td>37 (29)</td>
<td>NS</td>
</tr>
<tr>
<td>Age, y</td>
<td>61.3 (26–80.6)</td>
<td>63 (20–87)</td>
<td>NS</td>
</tr>
<tr>
<td>Drug therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diuretics</td>
<td>None</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>β-Blockers</td>
<td>None</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>None</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>Pathogenesis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic cardiomyopathy</td>
<td>...</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>...</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Hypertensive cardiomyopathy</td>
<td>...</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Valvular disease</td>
<td>...</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>NT proBNP levels, fmol/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>21.4 (5.7–991.9)</td>
<td>657 (6–29 368)</td>
<td>0.001</td>
</tr>
<tr>
<td>Male</td>
<td>12.5 (5.7–631.2)</td>
<td>464 (6–25 182)</td>
<td>0.001</td>
</tr>
<tr>
<td>Female</td>
<td>47.7 (5.7–991.9)</td>
<td>3127 (104–29 368)</td>
<td>0.001</td>
</tr>
<tr>
<td>UTN levels, fmol/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>6.6 (3.1–42.6)</td>
<td>22.1 (3.1–49.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>Male</td>
<td>7.2 (3.1–42.6)</td>
<td>22.4 (3.1–46.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>Female</td>
<td>4.6 (3.1–17.4)</td>
<td>20.6 (3.1–49.2)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Medians (ranges) are reported unless otherwise indicated. P values were computed using the Mann-Whitney test (comparing control and heart failure patients).

The Table illustrates the characteristics of the control and HF patients, who were well matched for age and sex. N-BNP was significantly elevated in HF patients. In control subjects, there was a correlation of N-BNP with age (r=0.41, P<0.001; data not shown), and women had higher levels than.
Men (P<0.001; Table). N-BNP increased with New York Heart Association (NYHA) class (Figure 1B; P<0.001 by Kruskal Wallis test). Plasma UTN was also elevated in HF patients (Table), but there was no correlation with age (data not shown), and levels were lower in women compared with men (P<0.001, Table). Plasma UTN was unchanged with increasing NYHA class (Figure 1B). N-BNP and UTN were elevated in HF patients irrespective of sex (P<0.001). N-BNP and UTN were modestly correlated (Spearman’s rho 0.35, P<0.001). UTN was not correlated to systolic function on echocardiography (P not significant).

With the general linear model procedure, analysis of N-BNP levels in HF patients yielded an r² of 0.446 for a model (P<0.001) with age, sex, and NYHA class as significant predictors (P<0.034, 0.002, and 0.001, respectively). None of these factors were identified as predictors of UTN (r²=0.058). Thus, UTN levels in HF patients were elevated irrespective of age, sex, or NYHA class.

In HF patients, plasma UTN was not dependent on use of medication. Receiver operating characteristic (ROC) curves for detection of all HF revealed areas of 0.90 and 0.86 for N-BNP and UTN, respectively (Figure 2A; P<0.001 compared with the diagonal). Equivalent ROC curve areas for severe HF (classes III and IV) were 0.98 and 0.83, respectively. ROC curves for the detection of NYHA class I HF yielded a higher area for UTN compared with N-BNP (0.88 versus 0.80, P<0.05, Figure 2B). With the use of logistic regression analysis for diagnosis of NYHA class I HF, a model combining both peptides accounted for a Nagelkerke r² of 0.61 (P<0.0005), and the ROC curve area from the predicted probabilities (0.95) exceeded that of either peptide alone (P<0.01, Figure 2B). Both peptides were significant (P<0.0005) independent predictors of NYHA class I HF with odds ratios of 2.88 and 1.52 for UTN and N-BNP, respectively (for 50% elevation of peptide level).

**Discussion**

Size fractionation of plasma extracts yielded one peak of UTN-like immunoreactivity. This is in contrast to multiple molecular weight forms of UTN that have previously been described in culture media from adrenocortical carcinoma cells. Our measurements of plasma UTN in control subjects contrast with other published work with smaller numbers of subjects. Matsushita et al reported undetectable levels, but the lower limit of the UTN assay was insensitive at ≈36 fmol/mL. Our values are in between those reported by Totsume et al (4.4 fmol/mL) and Wilkinson et al (12 fmol/mL). Some of these differences may be due to subject selection or assay performance.

The present study demonstrates that plasma UTN and N-BNP levels are elevated in HF. Because N-BNP and BNP are strongly correlated, similar findings would be expected for BNP. Although plasma N-BNP is elevated with age and female sex, such age trends are not evident for UTN. These age and sex differences in peptide measurements emphasize the importance of age and sex matching in studies. In contrast to N-BNP, where levels rise with NYHA class, UTN levels were elevated in NYHA class I patients, with no further change for more severe disease. Douglas et al reported UTN myocardial expression that showed a graded increase with disease severity. The source of UTN in plasma is currently unknown but may be cardiovascular in origin, because of the documented increased expression in cardiomyocytes, endothelium, and vascular myocytes. The contribution from cardiac and extracardiac sites may vary with disease severity, which provides a possible explanation for the discrepancy of our findings in plasma to that of myocardium. It is also currently unclear whether the increased level is causal to or an effect of ventricular dysfunction.

For the detection of HF, the area under the ROC curve for UTN is comparable to that of N-BNP, indicating a possible use for the peptide in accurate diagnosis of HF. This is especially true for diagnosis of mild HF (NYHA class I), and a combination of both peptides may be most useful. A potentially useful feature of UTN for diagnosis of HF is its independence of factors such as age, sex, and NYHA class. N-BNP, in contrast, is significantly affected by these variables.

The involvement of this new cardiovascular peptide in HF pathophysiology suggests an additional hormonal system that
could in the future be modulated with appropriate receptor ligands. Its use as a diagnostic test for HF may be more immediate.

Acknowledgments
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References
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