Heart is the Target Organ of Endogenous Cardiac Natriuretic Peptides

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Summary
This study aimed to evaluate whether the heart is the target organ of endogenous atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) in patients with heart failure (HF) with reduced ejection fraction (HFrEF).

We measured the plasma levels of cyclic guanosine monophosphate (cGMP), which is a second messenger of ANP and BNP, in the aortic root (AO) and coronary sinus (CS) in 237 patients with HFrEF. Plasma levels of cGMP were significantly higher in the CS than those in the AO in 237 patients with HFrEF (10.0 ± 4.5 versus 10.5 ± 4.3 pmoL/mL, P < 0.0001) and were significantly higher in the CS than those in the AO (8.0 ± 3.6 versus 8.9 ± 3.8 pmoL/mL, P < 0.0001) in mild HF patients (New York Heart Association (NYHA) II, n = 114), but there was no difference in plasma cGMP between the AO and the CS (11.9 ± 4.4 versus 11.9 ± 4.3 pmoL/mL, NS) in severe HF patients (NYHA III-IV, n = 123). In mild HF patients, log (ANP + BNP) in the AO was an independent predictor of (CS-AO) cGMP among hemodynamics and nitrate therapy. There was a significant correlation between log [(CS-AO) ANP + (CS-AO) BNP] and (CS-AO) cGMP (r = 0.455, P < 0.0001) in mild HF patients.

These findings indicate that cGMP is produced from the failing heart and that the heart is the target organ of endogenous ANP and BNP in patients with HFrEF. In severe HF patients, cGMP production may be attenuated because of the downregulation of biological receptors and/or increased cGMP degradation in the failing heart.

Key words: Atrial natriuretic peptide, Brain natriuretic peptide, Cyclic guanosine monophosphate, Heart failure, Heart failure with reduced ejection fraction

After discoveries of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), many translational studies have been reported, and plasma levels of BNP and N-terminal pro-BNP, as a marker of ventricular wall stress, are well-established biomarkers in patients with heart failure (HF). Despite dramatic increases in circulating ANP and BNP concentrations as HF progresses, their effects become blunted in patients with advanced HF. Previous studies including endogenous ANP and BNP infusion could not improve prognoses in patients with acute decompensated HF partly due to downregulation of biological receptors of ANP and BNP. However, the plasma level of transcardiac cyclic guanosine monophosphate (cGMP), which is a second messenger of ANP, BNP, and nitric oxide, remains unknown.

After angiotensin receptor neprilsyin inhibitor (ARNI) treatment in patients with HF with reduced ejection fraction (HFrEF), cardiovascular death decreased by 20% compared with that after enalapril, with increases in endogenous BNP and cGMP. Augmented plasma levels of ANP, mainly secreted from the atrium, and BNP, mainly secreted from ventricle by ARNI, may improve mortality in mild HF patients. Interestingly, not only HF but also sudden death was significantly reduced by ARNI under standard HF treatment suggesting that the activation of ANP, BNP, and C-type natriuretic peptide (CNP) may improve sudden death via the biological receptors in the failing heart.

We hypothesized that the heart is not only the source but also the target organ of endogenous ANP and BNP in patients with HFrEF and that transcardiac increase of cGMP is attenuated in severe HF patients compared with mild HF patients.

Methods
Patients: The subjects were HFrEF patients, which was defined as a left ventricular ejection fraction (LVEF) < 40%, who underwent cardiac catheterization for different
clinical indications (ischemic heart disease; for diagnosis and/or reevaluation of coronary artery stenosis, and non-ischemic heart disease and for diagnosis of dilated cardiomyopathy or hypertensive heart disease) from 1999 to 2011. LVEF was measured by two-dimensional echocardiography or ventriculography using contrast medium or radionuclide before the study. Etiologies of HFrEF were ischemic cardiomyopathy, dilated cardiomyopathy, or hypertensive heart disease. Patients with a low BNP level (BNP < 100 pg/mL) at cardiac catheterization were excluded. Estimated glomerular filtration rate (eGFR) was used as an indicator of renal function based on the abbreviated Modification of Diet in Renal Disease study formula, and patients with low eGFR (< 30 mL/minute/1.73 m²) were excluded. Patients with acute coronary syndrome, hypertrophic cardiomyopathy, congenital heart disease, valvular heart disease, primary pulmonary hypertension, lung disease, or pacemaker implantation and those on dialysis therapy were also excluded. The New York Heart Association (NYHA) functional class was evaluated on the day of cardiac catheterization. Informed consent was obtained from all patients for participation in the study, according to a protocol approved by the Committee on Human Investigation at our institution.

Study protocol: All patients were premedicated with an oral dose of diazepam (5 mg) and rested in bed in a supine position for at least 20 minutes. Left-sided cardiac catheterization was performed, and blood pressure was measured. Blood samples for measuring plasma levels of ANP, BNP, and cGMP were collected simultaneously from the aortic root (AO) and coronary sinus (CS). A 6 Fr catheter for blood sampling was positioned in the CS, and the position of the catheter was confirmed as previously reported.20-23) Samples for the assay of plasma ANP, BNP, and cGMP concentrations were transferred to chilled disposable tubes. The blood samples were immediately placed on ice and centrifuged at 4°C, and the plasma was frozen in aliquots and stored at −30°C until assay. Plasma concentrations of ANP and BNP were measured with a specific immunoradiometric assay for human ANP and BNP using a commercial kit (Shionogi, Japan) as previously reported.20-23) and plasma cGMP levels were measured using a sandwich immunoassay (Yamasa Shoyu, Japan) as previously reported.2,22,24) Statistical analysis: All results are expressed as the mean ± SD or median (interquartile range). Categorical data were compared by chi-squared distribution. Univariate analysis was performed using Student’s t-test. Because the ANP and BNP levels were not normally distributed, differences between the groups were analyzed by Mann–Whitney U-test, and differences in mean levels of AO and CS were tested by the nonparametric Wilcoxon rank-sum test for paired values. To evaluate the contribution of (CS-AO) ANP + (CS-AO) BNP and (CS-AO) cGMP (pmol/mL) in mild HF patients (r = 0.445, P < 0.0001) (Figure 3A). There was no correlation between log [(CS-AO) ANP + (CS-AO) BNP] (pmol/mL) and (CS-AO) cGMP (pmol/mL) in mild HF patients (r = 0.132, P = 0.149) in severe HF patients (Figure 3B).

Discussion

The present study for the first time evaluated the plasma levels of cGMP, which is a second messenger of ANP, BNP, and nitric oxide, in the AO and CS in patients with HFrEF. Our results indicated that the heart is not only the source but also the target organ of ANP and BNP in patients with HFrEF. Both ANP and BNP are cardiac in origin. Their highest levels are in the CS, and ANP is mainly secreted from the atrium and BNP mainly from the ventricle, and therefore, plasma ANP in the AO binds biological receptors though coronary circulation in the failing ventricle. Judging from the transcardiac increase of cGMP in mild HF patients, together with the significant correlation between log [(CS-AO) ANP + (CS-AO) BNP] and (CS-AO) cGMP (Figure 3A), the heart may be the target organ of ANP and BNP. In addition, both ANP and BNP may also act in an autocrine and paracrine manner in the heart. In severe HF patients, cGMP production may possibly be attenuated because of the downregulation of these biological receptors and/or increased cGMP degradation in the failing heart25) and/or endothelial dysfunction of coronary circulation.
We previously measured the plasma levels of ANP, BNP, and cGMP in the femoral artery and vein in patients with CHF. The cGMP production in both pulmonary and peripheral circulation was attenuated in severe CHF patients despite increases of ANP and BNP. These results may indicate the downregulation of pulmonary and peripheral vascular beds in which biological receptors exist, and these patients had poor prognosis. Taken together with the present study, biological receptors in human heart tissue including vascular beds and the failing myocardial tissue may also be downregulated. Exogenous ANP and BNP infusion could not improve prognoses in patients with acute decompensated HF; the mechanism of the prognostic improvement of LCZ696 in CHF patients remains unknown. Judging from our present study, the prognostic impact of LCZ696 may associate with the increase (CS-AO) cGMP difference, a marker of cardioprotective effect via biological receptors, in mild HF patients. However, there was no significant increase of (CS-AO) cGMP in severe HF patients, suggesting these findings may associate with no beneficial effect on primary end point (cardiovascular death or HF hospitalization) of LCZ696 in severe CHF by subanalysis.

Although we could not evaluate the CNP, both endogenous ANP and BNP bind biological receptors in failing heart and may increase cGMP and protect against myocardial injury in patients with HFref. The beneficial effects of LCZ696 appear to be driven by ANP rather than BNP, because of the 4-fold increase in ANP with no change in BNP after LCZ696. If, so, increased ANP from the atrium in the AO after LCZ696 binds biological receptors in coronary circulation in the failing ventricle.

The plasma cGMP level may be regulated by particu-

| Table 1. Patient Characteristics According to Severity of HF in Patients with HFrEF |
|------------------|------------------|------------------|------------------|------------------|
|                   | Total Group      | Mild HF          | Severe HF        | P value          |
|                   | (n = 237)        | (n = 114)        | (n = 123)        |                  |
| Age, years        | 64.9 ± 12        | 64.9 ± 12        | 64.9 ± 13        | NS               |
| Male, n (%)       | 180 (76)         | 93 (82)          | 87 (71)          | 0.067            |
| BMI (kg / m²)     | 22.7 ± 3.7       | 22.8 ± 3.7       | 22.7 ± 3.7       | NS               |
| NYHA class, n (%) |                  |                  |                  |                  |
| II                | 114 (48)         | 114 (100)        |                  |                  |
| III               | 98 (41)          | 98 (80)          |                  |                  |
| IV                | 25 (11)          | 25 (20)          |                  |                  |
| Etiology of heart failure |  |                  |                  |                  |
| Ischemic cardiomyopathy | 127 (54)       | 73 (64)          | 54 (44)          | 0.002            |
| Dilated cardiomyopathy | 81 (34)        | 28 (25)          | 53 (43)          | 0.003            |
| Hypertensive heart disease | 29 (12)        | 13 (11)          | 16 (13)          | NS               |
| Hypertension, n (%) | 123 (52)        | 60 (53)          | 63 (51)          | NS               |
| Diabetes mellitus, n (%) | 66 (28)        | 39 (34)          | 27 (22)          | 0.04             |
| Heart rate (beats/minute) | 70.8 ± 16       | 69.2 ± 14        | 72.2 ± 17        | NS               |
| Mean blood pressure (mmHg) | 86.3 ± 16       | 86 ± 15          | 86.5 ± 17        | NS               |
| LVEF (%)          | 28.2 ± 6.9       | 29.8 ± 6.2       | 26.7 ± 7.2       | 0.0006           |
| LVEDP (mmHg)      | 16.4 ± 6.1       | 12.9 ± 4.7       | 19.7 ± 5.5       | < 0.0001         |
| Creatinine (mg / dL) | 0.96 ± 0.28     | 0.96 ± 0.17      | 0.96 ± 0.29      | NS               |
| eGFR (mL/minute/1.73 m²) | 62.5 ± 21      | 63.1 ± 26        | 61.8 ± 22        | NS               |
| Hemoglobin (g/dL) | 12.6 ± 2.0       | 12.7 ± 2.1       | 12.5 ± 2.0       | NS               |
| ANP in the AO (pmol/mL) | 33.6 (21, 59) | 22.9 (17, 33) | 54 (31, 73) | < 0.0001 |
| ANP in the CS (pmol/mL) | 194 (112, 344) | 168 (105, 247) | 233 (134, 421) | < 0.0001 |
| (CS-AO) ANP (pmol/mL) | 164 (82, 931) | 141 (243, 307) | 188 (86, 223) | < 0.05 |
| BNP in the AO (pmol/mL) | 63.5 (44, 99) | 46 (36, 63) | 94 (62, 158) | < 0.0001 |
| BNP in the CO (pmol/mL) | 161 (113, 273) | 137 (101, 195) | 208 (133, 355) | < 0.0001 |
| (CS-AO) BNP (pmol/mL) | 100 (86, 341) | 95 (54, 137) | 106 (68, 200) | < 0.01 |
| cGMP in the AO (pmol/mL) | 10.0 ± 4.5 | 8.0 ± 3.6 | 11.9 ± 4.4 | < 0.0001 |
| cGMP in the CS (pmol/mL) | 10.5 ± 4.3 | 8.9 ± 3.8 | 11.9 ± 4.3 | < 0.0001 |
| (CS-AO) cGMP (pmol/mL) | 0.41 ± 1.4 | 0.86 ± 1.4 | −0.01 ± 1.2 | < 0.0001 |

BMI indicates body mass index; NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; LVEDP, left ventricular end-diastolic pressure; eGFR, estimated glomerular filtration rate; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; cGMP, cyclic guanosine monophosphate; AO, aortic root; CS, coronary sinus; HF, heart failure; HFref, heart failure with reduced ejection fraction; ACEI, angiotensin-converting enzyme inhibitor; and ARB, angiotensin receptor blocker.
late guanylyl cyclase, present in biological receptors of ANP, BNP, and CNP, and also may be regulated by soluble guanylyl cyclase and nitric oxide. In the present study, we included log ANP in the AO, log BNP in the AO, LVEF, LVEDP, age, sex, eGFR, nitrate treatment and log (ANP + BNP) in the AO to evaluate transcardiac increase in cGMP (Table II). We previously reported that the nitroglycerin infusion increased plasma arteriovenous cGMP difference; therefore, nitrate treatment included variables for the increase of cGMP production. However, no impact of nitrate treatment on the cGMP level was seen in the present study, suggesting that the source of cGMP in plasma may be mainly by a particulate guanylyl cyclase.

**Limitations:** Although we have reported several studies, using a database in our institution, there were several limitations to this study. The study populations may be heterogeneous partly due to catheter indication for the study period (1999 to 2011). This study was analyzed retrospectively after the publication of the results of LCZ 696. The mean LVEF was 28.2%, and relatively small number of patients receiving ACE-I or ARB and β-blocker may due to the study period. However, if the patients who did not receive ACE-I or ARB treatments were excluded, the results were similar (data not shown). There has been no previous study into the clearance of cGMP from the plasma, nine variables including were selected ANP, BNP, and the others including eGFR. The balance of production and clearance of cGMP just, like ANP and BNP, regulates plasma cGMP levels in the AO and the CS. We evaluated the (CS-AO) cGMP; this parameter may not be influenced by renal function but regulated by local regulation of the heart and coronary circulation such as endothelial function, because coronary vascular dysfunction has an important role in the prognosis of HF. ARNI has not yet been approved for use in Japan. After the approval of ARNI, the measurements of cGMP in the AO and CS before and after ARNI may support our hypothesis.

In conclusion, the transcardiac increase of cGMP suggests that the heart is not only the source but also the target organ of endogenous ANP and BNP in patients with HFrEF. In severe HF patients, cGMP production may be attenuated because of the downregulation of biological receptors and/or increased cGMP degradation in the failing heart.

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Table II. Univariate and Multivariate Linear Models of Transcardiac Increase in cGMP in Mild HF Patients

| Variables      | Univariate correlation coefficient | $P$ value | Multivariate beta-coefficient (SE) | $P$ value |
|----------------|-----------------------------------|-----------|------------------------------------|-----------|
| Age (years)    | 0.011                             | 0.871     |                                    |           |
| Gender (male = 1) | 0.076                             | 0.245     |                                    |           |
| Nitrate treatment (yes = 1) | 0.056                             | 0.395     |                                    |           |
| eGFR (mL/minute/1.73 m$^2$) | 0.023                             | 0.727     |                                    |           |
| LVEDP (mmHg)  | 0.047                             | 0.475     |                                    |           |
| LVEF (%)      | 0.029                             | 0.659     |                                    |           |
| Log ANP in the AO (pmoL/mL) | 0.248                             | 0.0001    |                                    |           |
| Log BNP in the AO (pmoL/mL) | 0.175                             | 0.0072    |                                    |           |
| Log (ANP + BNP) in the AO (pmoL/mL) | 0.265                             | $<0.0001$ | 0.0014 (0.0003)                   | $<0.0001$|

Abbreviations are listed in Table I.

Figure 3. A: Correlation between log [(CS-AO) ANP + (CS-AO) BNP] (pmoL/mL) and (CS-AO) cGMP (pmoL/mL) in mild HF patients. B: Comparison between log [(CS-AO) ANP + (CS-AO) BNP] (pmoL/mL) and (CS-AO) cGMP (pmoL/mL) in severe HF patients. AO indicates aortic root; CS, coronary sinus; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; cGMP, cyclic guanosine monophosphate; and HF, heart failure.

Disclosure

Conflicts of interest: None.

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