Elevated Plasma Levels of Adropin in Heart Failure Patients

Wenlin Lian, Xiaosong Gu, Yongwen Qin and Xing Zheng

Abstract

Background  Recent studies have suggested that a higher body mass index (BMI) is associated with an improved prognosis in heart failure (HF). Adropin is a recently identified protein that has been implicated in the maintenance of energy homeostasis. In the present study, we investigated plasma adropin levels in patients with HF and evaluated the relationship between the levels and the severity of HF.

Methods and Results  The study group comprised 56 patients with HF and 20 control subjects, who were divided into 4 subgroups according to New York Heart Association (NYHA) functional classification. Plasma levels of adropin, brain natriuretic peptide (BNP) and cardiac hemodynamics were determined. Plasma adropin levels were significantly increased according to the severity of NYHA class in the patients with HF; control: 6.0 ± 0.3; NYHA functional Class II: 7.6 ± 0.4; NYHA functional Class III: 9.8 ± 0.5; NYHA functional Class IV: 12.4 ± 0.6 ng/mL (p<0.01). Similarly, plasma BNP levels were significantly increased in accordance with the NYHA class. Plasma adropin levels were correlated positively with BNP (r=0.723, p<0.001), interleukin-6 (IL-6) (r=0.326, p=0.007) and body mass index (BMI) (r=0.295, p=0.014), and negatively with left ventricular ejection fraction (LVEF) (r=-0.710, p<0.001).

Conclusion  Plasma adropin levels were significantly increased according to the severity of HF, and BNP and BMI had independent impact on the plasma adropin level. These findings suggest that the augmented release of adropin may be involved in the pathogenesis of HF and further study is necessary to explain the precise role of adropin in HF.

Key words: adropin, heart failure, brain natriuretic peptide

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Introduction

Obesity is a known risk factor for cardiovascular disease and for the development of heart failure (HF) (1). However, recent data suggest that high body mass index (BMI) is associated with a more favorable prognosis in patients with established HF (2-5). The mechanisms underlying this finding remain unexplained, but negative energy balance and subsequent weight loss may be of importance (6).

Adropin is a recently identified protein encoded by the energy homeostasis-associated gene (Enho) that is expressed in the liver and the brain (7). The expression of adropin is regulated by energy status and dietary nutrient content, and is altered with obesity (7). In diet-induced obese mice, transgenic overexpression or systemic administration of adropin markedly attenuated insulin resistance and glucose intolerance, both key components of the metabolic stress response (7). Hence, adropin may be involved in the mechanisms by which body mass and body composition affect the prognosis in HF. To date, adropin has not been studied in patients with HF, and consequently, the possible role of adropin in relation to HF severity is unknown.

It is well-known that brain natriuretic peptide (BNP) increases with the severity of left ventricular dysfunction, and high values of BNP are associated with a poor prognosis (8). In addition, the plasma level of BNP appears to be inversely associated with BMI (9). The expression of adropin is altered with obesity, we hypothesized that a relationship between plasma levels of adropin and BNP exists.
To test this hypothesis, we investigated plasma adropin levels in patients with HF and evaluated the relationship between the levels and the severity of HF.

Subjects and Methods

Subjects

Enrolled in the present study were 56 consecutive HF patients (23 men and 33 women; mean age 76.8 ± 7.6 years, range 60-92 years) who were admitted to Changhai Hospital either for the treatment of worsening HF, or for the diagnosis and treatment of HF between April 2009 and April 2010. The diagnosis of HF was based on a history of dyspnea and symptomatic exercise intolerance, the presence of pulmonary congestion and/or evidence of impaired left ventricular systolic function. Impaired left ventricular systolic function was defined as a left ventricular ejection fraction (LVEF) of less than 40%, which was assessed by echocardiography. The patients were divided into 3 subgroups according to New York Heart Association (NYHA) class: NYHA II, 20 patients; NYHA III, 18 patients; NYHA IV, 18 patients. Twenty healthy persons (9 men and 11 women; mean age 72.4 ± 8.4 years, range 57-82 years) who underwent routine health checkups in the same hospital were used as controls. Age, gender, and body mass index (BMI) of the control subjects were similar with those of the HF patients.

Exclusion criteria were patients with primary valvular heart disease, uncontrolled hypertension, peripheral vascular disease, chronic obstructive pulmonary disease, immunosuppressive therapy, renal failure, and/or musculoskeletal conditions limiting exercise capacity such as rheumatic arthritis.

The protocol of the present study was performed according to the principles of the Declaration of Helsinki and approved by the local research and ethics committee of Changhai Hospital, Shanghai, China. Written informed consent was obtained from all subjects before participation.

Hormonal assay

After a minimum 8-hour overnight fast and 20 minutes of supine rest, venous blood was drawn into EDTA tubes and promptly centrifuged at 4°C, and plasma was frozen at -80°C in aliquots until analyses of adropin, brain natriuretic peptide (BNP), tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) were performed. The plasma IL-6 and TNF-α were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Jiamay Biotech Co, Changsha, CN and Majorbio Biotech Co, Shanghai, CN, respectively). BNP was determined by radioimmunoassay (RIA) kit (Phoenix Pharmaceuticals, Belmont, CA, USA).

The plasma adropin levels were measured using a commercial ELISA kit (Cusabio Biotech Co, Wuhan, CN) according to the manufacturer’s instructions. The assay recognizes recombinant and natural human adropin. No significant cross-reactivity or interference was observed. Test range was 0.32-20 ng/mL. The sensitivity of the assay was 0.08 ng/mL, and interassay and intraassay coefficients of variation (CV) were less than 14% and 5%, respectively.

Fasting plasma glucose (FBG), lipids, creatinine (Cr) and blood urea nitrogen (BUN) were measured in the Clinical Laboratory of Changhai Hospital. FBG was measured by an automated glucose oxidase method (Automatic Analyzer 2700, Olympus, Japan). Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) were measured by enzymatic methods using an autoanalyzer.

Statistical analysis

All values were expressed as the mean ± SEM. Continuous variables were analyzed by one-way ANOVA or the Kruskal-Wallis test to compare the difference among the 3 or 4 groups. For regression analysis, log transformation was used to normalize the distribution of plasma levels of BNP and TNF-α. Simple linear regression analysis was performed to calculate correlation coefficients. Multiple regression analysis was applied to determine the independent relation of clinical parameters (BMI, BNP, Cr, BUN, TC, LDL-C, HDL-C, TG, BNP, IL-6, TNF-α, LVEF) with plasma adropin level. For all analyses, a two-tailed p-value <0.05 was considered statistically significant. All of the analyses were performed using SPSS for Windows (version 10.0; SPSS Inc., Chicago, IL, USA).

Results

Subjects

The clinical features of both the patients with HF and the control subjects according to NYHA functional class are described in Table 1. The LVEF gradually decreased and, inversely, plasma levels of BNP were exponentially elevated according to NYHA class in the HF patients. Plasma BNP had a negative correlation with LVEF (r=-0.889, p<0.001). Plasma levels of IL-6 and Cr were significantly higher in the Class IV patients than those in the control group (p=0.032, p=0.026, respectively). Lipid analysis showed that plasma levels of TC and LDL-C were lower in the Class IV than those in the control group (p=0.005, p=0.030, respectively).

Plasma adropin in different NYHA functional classes

The plasma adropin levels in both the patients with HF and the control subjects are shown in Fig. 1. The plasma level of adropin increased with the deterioration of cardiac function (control: 6.0 ± 0.3 ng/mL; NYHA functional Class II: 7.6 ± 0.4 ng/mL; NYHA functional Class III: 9.8 ± 0.5 ng/mL; NYHA functional Class IV: 12.4 ± 0.6 ng/mL, respectively, p<0.001).

Correlations of adropin with various parameters

Table 2 and Fig. 2 show the correlation between plasma adropin level and several clinical parameters in the patients.
with HF. Plasma adropin level had a positive correlation with plasma BNP levels (r=0.723, p<0.001), plasma IL-6 levels (r=0.326, p=0.007), plasma Cr (r=0.238, p=0.039) and BMI (r=0.295, p=0.014). A correlation between plasma levels of adropin and BNP with log transformation of these values is shown in Fig. 2. Plasma adropin levels were negatively correlated with LVEF (r=-0.710, p<0.001). In the multiple regression analysis, plasma BNP and BMI had an independent impact on plasma adropin level in patients with HF (Table 3).

**Association between adropin levels and final diagnosis**

The capacity of adropin to differentiate heart failure patients from health people was assessed with a receiver operating characteristic curve analysis (Fig. 3). The area under the receiver operating characteristic curve when adropin was used to differentiate heart failure patients from health people was 0.80 (95% confidence interval, 0.70-0.89; p<0.001). An adropin cutoff value of 7.0 ng/mL had a sensitivity of 73% and a specificity of 75% for differentiating heart failure patients from health people.

**Discussion**

The key findings from the present study were that there were significant differences in the plasma adropin level between HF patients with different NYHA functional classes and control subjects. Moreover, the plasma adropin level increased according to the NYHA functional class. To the best of our knowledge, this is the first report to describe the disturbance of plasma adropin in patients with HF.

The present study showed that the plasma adropin level had a positive correlation with the plasma level of BNP and a negative correlation with LVEF. BNP is synthesized in the ventricular myocardium, and both BNP and LVEF indicate the severity of heart failure (8, 10). The elevated level of plasma BNP could have a diuretic and a natriuretic action in the kidney and produce vasodilation in the vascular beds, which may help preserve cardiac function in HF. We also considered the possible pathophysiological role of adropin in HF patients. Lovren et al recently showed that adropin is also expressed in human umbilical vein and coronary artery endothelial cells (ECs) and has effects to regulate EC function via upregulating endothelial nitric oxide synthase.

![Figure 1. Plasma adropin levels in the patients with heart failure according to New York Heart Association (NYHA) functional class (p-value for Kruskal-Wallis H test, p<0.001).](image-url)

Table 2. Correlation between Plasma Adropin Level and Various Clinical Parameters in the Patients with Heart Failure

<table>
<thead>
<tr>
<th>Parameter</th>
<th>r</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adropin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs BMI</td>
<td>0.295</td>
<td>0.014</td>
</tr>
<tr>
<td>vs Total cholesterol</td>
<td>-0.141</td>
<td>0.150</td>
</tr>
<tr>
<td>vs LDL–cholesterol</td>
<td>-0.159</td>
<td>0.122</td>
</tr>
<tr>
<td>vs HDL–cholesterol</td>
<td>-0.190</td>
<td>0.081</td>
</tr>
<tr>
<td>vs Triglyceride</td>
<td>0.142</td>
<td>0.148</td>
</tr>
<tr>
<td>vs BNP</td>
<td>0.723</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>vs IL–6</td>
<td>0.326</td>
<td>0.007</td>
</tr>
<tr>
<td>vs TNF–α</td>
<td>0.040</td>
<td>0.384</td>
</tr>
<tr>
<td>vs LVEF</td>
<td>-0.710</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>vs Blood urea nitrogen</td>
<td>0.168</td>
<td>0.108</td>
</tr>
<tr>
<td>vs Creatinine</td>
<td>0.238</td>
<td>0.039</td>
</tr>
<tr>
<td>vs Fasting glucose</td>
<td>-0.117</td>
<td>0.195</td>
</tr>
</tbody>
</table>

n = 56
Abbreviations see in Table 1.
Log transformation was used to normalize the distribution of plasma levels of BNP and TNF–α.

Figure 2. Relationship of plasma adropin level with plasma level of brain natriuretic peptide (BNP) in patients with heart failure (r=0.728, p<0.001). Log transformation was used to normalize the distribution of the plasma levels of BNP (n=56).

(eNOS) (11). Endothelial dysfunction (ED) has been widely reported in patients with HF (12). ED is able to aggravate the failing heart function since it leads to the higher peripheral resistances observed in HF. Thus, an increased afterload and myocardial microcirculation abnormalities participate in the development of cardiac damage and decompensation (13). In turn, left ventricular impairment may negatively impact endothelial function by reducing shear stress and vascular nitric oxide (NO) bioavailability (14). Taken together, these findings suggest that adropin is as a functional link between EC and HF, and might decelerate left ventricular dysfunction in HF. On the other hand, in humans the natriuretic peptides are involved in fat metabolism as a stimulant for lipolysis (15), thus it could be hypothesized that lipolysis by BNP is associated with adropin synthesis in HF. Further studies are necessary to elucidate the precise role of adropin in HF.

The present study also showed that BMI had an independent impact on plasma adropin level. Cardiac cachexia, a state characterized by weight loss, muscle wasting and cytokine activation, occurs in patients with advanced HF (16). Though we did not directly evaluate cardiac cachexia in the present study, the changes in TC, LDL-C, HDL-C and TG among the different NYHA classes could indicate a wasting condition. Similarly, the BMI of patients with NYHA IV was lower than for the others, but the difference was not statistically significant. Kumar et al reported that liver Enho expression decreases with diet-induced obesity or with genetically induced obesity (7). Thus, we hypothesize that cardiac cachexia or wasting, which could reflect a decrease in visceral fat, and may contribute to the elevation of plasma adropin levels in patients with HF. However, we did not examine abdominal circumference and visceral fat because of the serious condition of the patients with HF on admission. Further study will be necessary to elucidate the precise mechanism for adropin release in patients with HF.

Our study had some limitations. In the present study, plasma adropin levels were significantly increased according to the severity of HF. However, whether the correlation between adropin and the severity of HF represents a true cause-and-effect relationship or just reflects parallel changes due to a common underlying cause in this population needs to be further clarified. Confirmation that the alteration of adropin is a major factor in the deterioration of HF will require investigating adropin transgenic overexpression or systemic treatment using recombinant protein of adropin in the model of HF.

In conclusion, the present study demonstrated that plasma adropin levels were significantly increased according to the severity of HF. Multiple regression analysis showed that BNP and BMI had an independent impact on plasma adropin level. These findings suggest that the augmented re-
The authors state that they have no Conflict of Interest (COI).

lease of adropin may be involved in the pathogenesis of HF, but further study is necessary to explain the precise role of adropin in HF.

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Table 3. Multiple Regression Analysis of Variable Associated with Plasma Adropin Level in the Patients with Heart Failure

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standardized coefficient</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adropin</td>
<td>vs BMI</td>
<td>0.225</td>
</tr>
<tr>
<td></td>
<td>vs BNP</td>
<td>0.475</td>
</tr>
<tr>
<td></td>
<td>vs LVEF</td>
<td>-0.279</td>
</tr>
<tr>
<td></td>
<td>vs Creatinine</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>vs IL–6</td>
<td>0.089</td>
</tr>
<tr>
<td></td>
<td>vs HDL–cholesterol</td>
<td>0.056</td>
</tr>
</tbody>
</table>

n = 56

Abbreviations see in Table 1.

Log transformation was used to normalize the distribution of plasma levels of BNP. The parameters listed were variables associated with plasma adropin levels at the p < 0.1 level in the simple linear regression analysis (Table 2).

Figure 3. Receiver-operating-characteristic curve for various cutoff levels of adropin in patients with heart failure and controls (n=76).

References


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