Reduced Apelin Levels in Stable Angina

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Abstract

Objective To investigate the change in the plasma apelin level in patients with stable angina.

Methods The study enrolled 96 patients with stable angina as the Stable Angina Group and another 78 outpatients with no angina as the Control Group. Subjects were excluded if they had a history of acute coronary syndrome, rheumatic heart disease, cardiomyopathy, cardiac arrhythmia, diabetes mellitus, hyperthyroidism, or antecedent hypertension. Plasma apelin levels of all subjects were determined using a commercially available immunoassay. In addition, blood was sampled for measurements of 8-iso-prostaglandin-F2alpha by enzyme-linked immunosorbent assay. The severity of coronary artery stenosis of stable angina patients was evaluated using the Gensini score.

Results The mean levels of apelin in plasma were significantly lower in subjects with stable angina compared with controls (1.24 vs. 1.98 ng/mL, p <0.05). The plasma level of apelin in the stable angina group was negatively correlated with the Gensini score (r =-0.399, p <0.05).

Conclusion Reduced apelin levels were observed in this homogenous population of stable angina subjects and the plasma apelin level was negatively correlated with the degree of coronary stenosis.

Key words: peptide, stenosis, coronary angiography, gensini score


Introduction

Apelin is a peptide recently isolated from bovine stomach extracts which appears to act as an endogenous ligand for the orphaned G-protein-coupled receptor (APJ) (1). It is strongly expressed in the heart, large conduit vessels, coronary vessels and endothelial cells (2). Recently, some cardiovascular functions of the apelin/APJ system have been described, such as endothelium-dependent vasodilatation, and vasoconstriction by direct action on the smooth muscle and positive inotropism (3, 4).

These findings indicated that apelin/APJ system might have certain pathophysiological roles in cardiovascular diseases. More recent reports have indicated that this peptide has been down-regulated in subjects with acute heart failure (5). Furthermore, apelin also induced vascular smooth muscle cell proliferation, which was inhibited by superoxide dismutase or dihydroxy iodonium; APJ deficiency is preventative against oxidative stress-linked atherosclerosis; the apelin-APJ system is a mediator of oxidative stress in vascular tissue (6), and thus we speculated it to have some relationship with arterial atherosclerosis.

Subjects and Methods

In total, 156 outpatients of China Medical University Hospital were admitted. The study enrolled 96 outpatients with stable angina (SA) as the SA group (n=96). Another 78 outpatients who came to the check-up center of China Medical University Hospital for physical examination and have not angina and coronary heart disease history or any symptoms was included as the control group (n=78). The study protocol was approved by the local ethics committee and informed consent was given by each patient before enrollment. Subjects were excluded if they had a history of acute coronary syndrome (ACS), rheumatic heart disease, cardiomyopathy, cardiac arrhythmia, diabetes mellitus, hyperthyroidism, or antecedent hypertension.

Blood sample collection for determination of fasting ape-
lin was drawn from all subjects after a minimum 12 hours overnight fasting. Blood samples obtained from subjects were kept in EDTA containing tubes and centrifuged. Plasma was extracted, aliquoted, and stored at -80°C until analysis. Plasma apelin-12 levels were determined using a commercially available enzyme immunoassay without extraction (Phoenix Pharmaceuticals, Burlingame, CA, USA) according to manufacturer’s instructions. This assay employs an immunoaffinity purified rabbit antibody specific for apelin 1-12. The antibody has 100% cross-reactivity to apelin 1-12, 1-13, and 1-36. The assay was performed in duplicate with intra-experimental standards using a Victor 3 plate reader (Perkin-Elmer, Wellesley, MA, USA). Values were normalized to a standard curve. The intra-assay and interassay variances for apelin-12 were 24 and 18%, respectively. Plasma levels of isoprostane 8-prostaglandin F2α (8-iso-PGF2α), an index of systemic oxidative stress (7), were determined by enzyme-linked immunosorbent assay using a commercial kit (Cayman Chemical Co., Ann Arbor, MI, USA).

Subjects in SA group underwent a coronary angiography using a commonly performed technique. The coronary angiograms were assessed by two experienced angiographers who used a validated quantitative coronary angiographic system (QCAMS5.1, MEDIS, Leiden, Netherlands). The degree of coronary artery stenosis was estimated using the Gensini score, which is computed by assigning a severity score to each coronary segment according to the degree of luminal narrowing and its geographic importance (8).

All results were expressed as the mean ± SE. Comparison of continuous variables between the two groups was performed using paired t test. Differences between groups for categorical variables were compared using an χ² test. Conditional logistic regression analysis was used to determine the relationship between apelin level and Gensini score. Multivariate analysis was performed to determine the correlates of apelin and clinical variables on Gensini score. A p value of less than 0.05 was considered statistically significant.

### Results

#### Baseline characteristics

During the study period, a total of 96 subjects with stable angina were enrolled. These subjects with stable angina were similar to 78 controls based on age and gender. Body mass index, left ventricular ejection fraction, and number of smokers were also similar between stable angina subjects and controls. In addition, plasma levels of 8-iso-PGF2α in SA group were higher than those in the control group. Detailed patients characteristics are shown in Table 1.

#### Level of plasma apelin and its relationship with Gensini score

The mean plasma apelin level was 1.24 ± 0.19 ng/mL in SA group and 1.98 ± 0.24 ng/mL in controls, respectively. Mean level of apelin was significantly lower in subjects with SA compared with controls (p<0.05) (Fig. 1). Coronary angiography characteristics of stable angina patients were analyzed by a validated quantitative coronary angiographic system by which we obtained the Gensini scores. Regression analysis revealed that the Gensini scores of the patients in SA group were inversely and significantly correlated with the plasma apelin level (r=-0.399, p<0.05) (Fig. 2).

#### Multivariate regression analysis for the Gensini score

Multiple regression analysis revealed that the plasma apelin level predicted the Gensini score independent of sex, age, hypertension, smoking and the lipid profiles, including the serum concentrations of total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) (Table 2). LDL-C and HDL-C concentrations also predicted the Gensini score in-
Apelin was first identified as a native ligand of the orphan G-coupled receptor APJ (9). This 13-amino acid peptide is cleaved from a pro-peptide by an unknown endopeptidase and then active at a number of sites throughout the body (10). Recent work has confirmed that apelin is a powerful vasodilator and positive inotrope, and it may be correlated with cardiovascular atherosclerosis (11). Therefore, we designed this study in order to investigate the possible relationship between apelin and coronary artery disease (CAD).

In the present study, the findings revealed that the levels of apelin were lower in stable angina group than in the control (1.24 vs 1.98 ng/mL). This result suggested that apelin may be involved in the pathophysiological process of coronary artery stenosis and agreed with the findings of a previous study that apelin may be a new plasma marker for cardiovascular disease in vivo (12). Moreover, conditional logistic regression analysis showed a significant and inverse relationship between the Gensini score and plasma apelin level (r = −0.399 p < 0.05). The higher Gensini score is accompanied by a lower apelin level. Gensini score is a measurement to estimate the extent of CAD (13), so our result suggested that apelin was associated with the extent of coronary stenosis. In multivariable analysis, the apelin level was significantly correlated with the Gensini score as well as HDL-C and LDL-C. This finding was consistent with previous reports that apelin/apj system played certain pathophysiological roles in vascular disease (14).

Since diabetes and atrial fibrillation were reported to be correlated with plasma level of apelin (15, 16), the study excluded diabetic and cardiac arrhythmia subjects to exclude confused factors. And many biological systems are altered during the process of ACS and may or may not affect the plasma levels of apelin, we excluded the patients with ACS in this study. Though the two groups in the present study were similar in age, sex, BMI, LVEF and number of smokers, it is not possible to completely eliminate the potential of confounding influence of other factors such as hypertension, hyperlipidemia, and medicines used by patients on apelin levels. Tasci and co-workers found that plasma apelin may be decreased in patients with elevated LDL-cholesterol (17). In our study, another limitation was the small number; the
number of patients with hyperlipidemia was slightly larger in stable angina than control group, which necessitates further study in future to exclude the influence of hyperlipidemia. To date, the relation of hypertension with apelin has not been defined. Kagiyama et al drew upon the conclusion that arterial blood pressure could be increased by apelin injection (18). While Mitra et al indicated that there was no reliable evidence that apelin can affect the blood pressure (19). Likewise, whether smoking, dietary, percutaneous coronary intervention or medication can affect the plasma apelin level has not been ascertained to date. In the final analysis, however, all these factors could affect our results and we need do more work to strengthen our conclusion. Even so, we believe that our study should contribute to the understanding of the association between apelin and coronary stenosis.

The mechanisms that reduce apelin level associated with severity of coronary artery stenosis have not been clarified. The biological roles of this peptide and others cleaved from the propeptide are only beginning to be explored (20, 21). The biological roles of this peptide and others cleaved from the propeptide are only beginning to be explored (20, 21). Recent work has confirmed that apelin is a powerful vasodilator and positive inotrope, and appears to be counter-regulated, at least to some extent, with the angiotensinogen pathway (22, 23). Apelin also may act centrally to counteract with the vasopressin pathway in the regulation of extracellular fluid volume (24). Another important view is that apelin directly activated the vascular L-Arg/NOS/NO pathway, which could be one of the important mechanisms of apelin-regulated vascular function in a physiological condition (25). In our study, the plasma levels of 8-iso-PGF\textsubscript{2α}

which serves as an index of systemic oxidative stress, were higher in the SA group than in the control group (472.6 \pm 74.8 vs 164.6 \pm 38.1 pg/mL). This result is in agreement with the view that apelin participates in the process of coronary atherosclerosis through the effects on lipid peroxidation which may be the reason why apelin levels were decreased in SA group.

Our study included a small number of patients, therefore the ability to generalize the correlation between apelin level and Gensini score might be limited. Our study was a cross-sectional study, not a randomized trial which may introduce biases to our analysis and may explain why we obtained a high correlation between the plasma apelin level and the Gensini score. Even though, to the best of our knowledge, this report is one of the few studies which indicates the possibility that plasma apelin levels could be correlated with the severity of coronary artery stenosis in humans. From the pathophysiological point of view, it might be speculated that apelin acts as a vasopressor in damaged vasculature (eg, atherosclerosis) and plasma apelin levels may be a useful indicator of the severity of coronary artery stenosis. In conclusion, the measurement of plasma apelin levels may be useful for predicting the severity of coronary artery stenosis.

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References


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