Plasma Level of Elabela in Patients with Coronary Heart Disease and Its Correlation with the Disease Classification

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Summary
This study aimed to evaluate the concentration of plasma elabela (ELA) in patients with coronary heart disease (CHD) and its correlation with the disease classification.

We enrolled 238 patients diagnosed by coronary angiography as CHD and 86 controls. The CHD group was divided into three subgroups: stable angina (SA), unstable angina (UAP), and acute myocardial infarction (AMI). The plasma levels of ELA were measured in all participants and compared among different groups. The relationship between ELA and CHD classification was analyzed.

ELA levels were markedly higher by 10.71% in patients with CHD than in controls (P < 0.05). The concentration of ELA in UAP and AMI subgroups were higher than in controls and SA subgroup. The former difference was significant (P < 0.05), but the latter was not. In addition, the ELA concentration was not correlated with SYNTAX score, left ventricular ejection fraction, and other biochemical variables.

The newfound hormone, ELA, significantly increased in patients with UAP and AMI. There is a tendency that ELA levels might be correlated with CHD classification, but not with lesion severity. ELA may play a role in acute coronary syndrome.

Key words: Bioactive peptide, Acute myocardial infarction, Acute coronary syndrome

Over the past few decades, coronary heart disease (CHD), with the increasing morbidity, has been seriously harming human health and has become a leading cause of death worldwide in modern society. There are many bioactive peptides in human cardiovascular system. Since the discovery of angiotensin domain type 1 receptor-associated proteins (APJ) receptor in 1993,1 increasing evidence showed that APJ is essential in embryonic and adult mammalian cardiovascular systems.2,3 Apelin is a ligand for APJ receptor found in 1998.4 According to papers, apelin and APJ receptor have some protective effects in myocardial infarction, heart failure, and pulmonary hypertension.3

Elabela (ELA), also named Toddler, is a newly discovered peptide that shares the APJ receptor with apelin.6 In animal models or in vitro studies, ELA can increase myocardial contractility and induced coronary artery dilation.7 Fe-ELA-21 fusion protein treatment promoted cardiomyocyte proliferation, increased angiogenesis, and reduced heart fibrosis.6 However, so far, there is only one clinical data on the association between ELA level and ST elevation myocardial infarction (STEMI).9

The present study was conducted to clarify the circulating ELA concentrations in patients with CHD and reveal the relationship with CHD and the disease classification.

Methods
Subjects: The study was reviewed and approved by the Ethics Committee of Peking University First Hospital. Subjects were recruited from patients who were admitted to our hospital from June 2017 to August 2018. Our sample included 324 patients (177 men; mean age, 66.52 ± 10.02 years old). All patients who underwent coronary angiography (CAG) for suspicious symptoms were divided into two groups: CHD, which included 238 patients with CHD, and control, which included 86 subjects with normal coronary artery. The CHD group was divided into three subgroups: stable angina (SA), which included 59 patients; unstable angina (UAP), which included 138 patients; and acute myocardial infarction (AMI), which included 41 patients. Diagnostic criteria for SA, UAP, and AMI were taken from the related guidelines from the

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American College of Cardiology/American Heart Association. CAG was performed in multiple views according to the standard Judkins technique. The SYNTAX score was used to define the severity of coronary lesions. The SYNTAX score was calculated in all patients by two experienced interventional cardiologists. The SYNTAX score was determined for all coronary lesions with > 50% diameter stenosis in a vessel of > 1.5 mm, based on the SYNTAX score calculator (www.syntax.com). Patients with acute infection, severe liver or renal disease, malignant tumor, and autoimmune diseases were excluded.

**Clinical examinations**: Blood samples were collected from the antecubital vein of subjects just before undergoing CAG for testing ELA and after an overnight fast for measuring other biochemical parameters. All subjects were asked about smoking, family history of disease, use of medications, and medical history. Subjects underwent physical examination, including height and weight measurement for the calculation of body mass index (BMI; kg/m²). Left ventricular ejection fraction (LVEF) was measured by ultrasonic cardiography.

Blood was drawn into tubes containing disodium ethylene diaminetetraacetic acid (1 mg/mL) and aprotinin (500 KIU/mL; Sigma, St. Louis, MO, USA) and then immediately centrifuged at 3500 g for 10 minutes at 4°C; plasma was stored at −80°C.

ELA was measured using an ELA kit (Phoenix Pharmaceuticals, USA). The minimum detectable concentration of ELA was 0.27 ng/mL. The intra- and inter-assay coefficient of variation was 10% and < 15%, respectively. The detection range was from 0.27 to 2.93 ng/mL.

The levels of triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), creatinine (Cr), uric acid (UA), high-sensitivity C-reactive protein (hsCRP), brain natriuretic peptide (BNP), glycosylated hemoglobin a1c (HbA1c) and homocysteine (HCY), creatine kinase-MB isoenzyme (CK-MB), and cardiac troponin I (cTnI) were assayed by routine automatic analysis.

**Statistical analysis**: The IBM SPSS Statistics version 14.0 was used for statistical analysis. Continuous variables with normal distribution were expressed as mean ± standard deviation, non-normal variables were reported as median (interquartile range), and categorical variables were expressed as percentages and/or numbers. Chi-square test and one-way ANOVA were used for statistical analysis. The correlation between variables was tested using the Pearson’s correlation coefficient. P < 0.05 was considered statistically significant.

**Results**

**Clinical characteristics**: The characteristics of the study subjects are listed in Table I. No significant differences were found between patients with CHD and controls in BMI, TG, TC, LDL, hsCRP, HCY levels, and LVEF. However, patients with CHD differed from controls in their significantly higher plasma levels of UA, Cr, BNP, and HbA1C (P < 0.05). There were more male patients and more smokers in the CHD group (P < 0.05). In addition, the HDL level of patients in the CHD group was much lower than in the controls (P < 0.05). There was no significant difference in complication with hypertension or family history. However, there were more patients using aspirin and clopidogrel in the CHD group (P < 0.05), and the differences of patients using β-blocker, calcium antagonists, and ACEI/ARB were not significant between the two groups (P < 0.05) (Table I).

**Plasma levels of ELA**: The level of ELA was markedly higher by 10.71% in patients with CHD (1.55 ± 0.23 ng/mL) than in controls (1.40 ± 0.15 ng/mL, P < 0.05) (Table II).

**Plasma levels of ELA, CK-MB, cTnI, and SYNTAX score in subgroups**: The concentrations of ELA in UAP (1.56 ± 0.24 ng/mL) and AMI (1.57 ± 0.21 ng/mL) subgroups were much higher than in controls (1.49 ± 0.24 ng/mL) (P < 0.05). There was a tendency that the levels of ELA in UAP and AMI subgroups were also higher than in SA subgroup (1.50 ± 0.21 ng/mL); however, the differences were not significant. The differences between the SA subgroup and controls and between the UAP and AMI subgroups were not significant.

SYNTAX scores in the SA, UAP, and AMI subgroups were much higher than in controls (P < 0.01). The concentrations of CK-MB and cTnI in the AMI subgroup were much higher than in controls and SA and UAP subgroups (P < 0.01) (Table III).

**Correlation of ELA with variables**: Univariate analysis revealed that plasma ELA was not correlated with age, gender, BMI, smoker, TG, TC, HDL, LDL, UA, Cr, hsCRP, HCY, BNP, HbA1c, and SYNTAX scores.

**Discussion**

This study demonstrated for the first time that plasma ELA levels increased in patients with CHD compared with controls. We also detected that there was a tendency that the concentration of ELA of patients in the UAP and AMI subgroups were higher than in controls and the SA subgroup. In addition, the concentration of ELA was not correlated with the severity of coronary artery lesions (SYNTAX scores).

ELA and apelin share a G-protein-coupled receptor named APJ. Apelin was the first isolated endogenous ligand of APJ. The apelin-APJ system plays numerous roles in the cardiovascular system, central nervous system, and many other systems. ELA was a newfound peptide ligand for APJ in 2013 by two independent teams. Human ELA gene consists of three exons on chromosome 4, which generates a transcript annotated as a noncoding RNA. ELA mRNA encodes a polypeptide of 54 aa. The molecular weight of the soluble mature peptide is less than 4 kDa. Just as scholars have speculated, ELA also played important roles in the cardiovascular system. ELA levels were decreased in patients with hypertension, and intravenous injection of ELA-21 significantly reduced the mean arterial pressure in spontaneously hypertensive rats. In addition, sustained ELA gene therapy may offer antihypertension and antirenal remodeling in salt-induced hypertension. ELA increased myocardial contractility and dilated coronary artery by the phosphorylation of ERK1/ERK2. In an animal test, the infusion of ELA...
proven cardiac dysfunction, hypertrophy, and fibrosis.\textsuperscript{15,16} In a recent report, Dönmez, \textit{et al.}\textsuperscript{7} have found that the ELA levels increased in patients with STEMI. In addition, they observed a moderate positive correlation between troponin I and ELA. Together, many reports supported that ELA played a protective effect in cardiovascular diseases.

In our study, ELA levels significantly increased in patients with acute coronary syndrome (ACS). Similar to the results of the work of Dönmez, \textit{et al.}, we do find that patients with AMI had much higher levels of ELA. However, the same pattern was also found in the UAP subgroup. This might be explained by the fact that the concentration of ELA increased in patients with CHD not only because of the necrotic myocardial tissue. At the state of ACS, including UAP and AMI, there were complicated pathophysiological changes. A lot of cytokines and metabolic pathways may get involved and may also influence the ELA levels. Perj\'s, \textit{et al.}\textsuperscript{17} found that ELA can affect cardiac contractility and cardiac output independent of protein kinase C. In addition, ELA dilated coronary arteries in mice by attenuating the potentiation of Ang II-induced vasopressor, which was NO-independent and slightly endothelium-dependent.\textsuperscript{17,18} The specific mechanism needs to be confirmed by further research. There are two types of AMI, STEMI and non-STEMI. Dönmez, \textit{et al.}\textsuperscript{7} have found that the ELA levels increased in patients with STEMI. Unfortunately, there were only nine patients with STEMI in our study, and determining if there were significant differences in ELA between the two types of AMI was difficult.

The cross-sectional design and subject size are the main limitations of our study. Thus, it was not clarified whether the increased ELA levels were the reason or result of CHD. More well-designed and large-scale studies are required to confirm the value of ELA in the cardiovascular system. In addition, the detailed mechanism also needs to be clarified.

**Conclusion**

The present study demonstrated for the first time that the plasma levels of ELA were increased in patients with ACS. There was a tendency that ELA levels might be correlated with the classification of CHD, but not with lesion severity, suggesting that ELA might contribute to the pathogenesis of ACS.

**Disclosure**

**Conflicts of interest:** None.
Table III. Elabela Levels in Controls, the Non-AMI Subgroup, and the AMI Subgroup

|          | Controls   | SA          | UAP          | AMI          |
|----------|------------|-------------|--------------|--------------|
| n        | 86         | 59          | 138          | 41           |
| Elabela (ng/mL) | 1.49 ± 0.24 | 1.50 ± 0.21 | 1.56 ± 0.24* | 1.57 ± 0.21* |
| SYNTAX   | 0 (0-0)    | 13.0 (5.0-20.0)** | 12.0 (7.0-20.6)** | 21.5 (9.5-29.3)** ΔΔ## |
| CK-MB (ng/mL) | 1.30 (1.00-2.00) | 1.30 (1.10-2.40) | 1.55 (1.20-2.30) | 4.60 (1.75-16.49)** ΔΔ## |
| cTnI (ng/mL)  | 0.004 (0.001-0.008) | 0.006 (0.001-0.011) | 0.006 (0.001-0.017) | 1.824 (0.438-4.296) ΔΔ## |

Data are mean ± SD or median (IQR). *P < 0.05 versus controls; **P < 0.01 versus controls; ΔΔP < 0.01 versus SA; ##P < 0.01 versus UAP. CK-MB indicates creatine kinase-MB isoenzyme; and cTnI, cardiac troponin I.

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