Plasma levels of Elabela are associated with coronary angiographic severity in patients with acute coronary syndrome

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Abstract

Background Elabela (ELA) was newly discovered as a novel endogenous ligand of the apelin receptor (APJ) which has demonstrated to be crucial for cardiovascular disease such as myocardial infarction, hypertension and heart failure. Previous experiments have revealed that ELA reduced arterial pressure and exerted positive inotropic effects on the heart. However, the role of plasma ELA levels in patients with acute coronary syndrome (ACS) and its relationship with severity of coronary arteries have not been investigated. Methods Two hundred and one subjects who were hospitalized for chest pain and underwent coronary angiography were recruited in this study. One hundred and seventy-five patients were diagnosed with ACS and twenty-six subjects with negative coronary angiography were included in the control group. Plasma ELA levels, routine blood test, blood lipid, liver and kidney functions were measured. The number of coronary arteries and SYNTAX (Synergy Between Percutaneous Coronary Intervention With Taxus and Cardiac Surgery) score of coronary lesions were used to evaluate the extent of coronary artery stenosis. Results ELA in patients with ACS was significantly higher than that in the control group (P < 0.01). There was no significant difference in plasma ELA levels among patients with single-, double- and triple-vessel diseases. However, in the generalized additive model (GAM), there was a threshold nonlinear correlation between the ELA levels and Syntax I score (P < 0.001). Plasma ELA levels were positively correlated with the Syntax I score when the ELA levels ranged from 63.47 to 85.49 ng/mL. There was no significant association between the plasma ELA levels and the extent of coronary artery stenosis when the ELA levels were less than 63.47 ng/mL or higher than 85.49 ng/mL. Conclusion The present study demonstrates for the first time that plasma ELA levels are increased in patients with ACS. The rise in endogenous ELA levels was associated with severity of coronary stenosis and may be involved in the pathogenesis of ACS.

Keywords: Acute coronary syndrome; Coronary artery stenosis; Elabela
cardiac infarction in animal models. Clinical research also showed increased ELA in patients with acute ST segment elevation myocardial infarction and a positive correlation among troponin I, NT-ProBNP and ELA. However, the role of ELA in the pathogenesis of atherosclerosis is not fully elucidated and has not been investigated.

Coronary atherosclerotic heart disease (CAD) is a growing public health problem and one of the main causes of death worldwide. The underlying mechanisms of CAD are complicated and involve a combination of endothelial dysfunction, inflammation, extensive lipid deposition in the intima, proliferation of vascular smooth muscle cells and activation of platelets. Apelin was shown to attenuate the progression of atherosclerosis by promoting cholesterol efflux and reducing foam cell formation. Some clinical studies found lower apelin levels in patients with established coronary artery diseases compared with controls. Evidence has shown that the ELA-APJ axis is a promising therapeutic target for cardiovascular diseases. In this study, we explored the relationship between plasma ELA levels and the severity of coronary artery stenosis in patients with acute coronary syndrome (ACS).

2 Methods

2.1 Study subjects

In this prospective study, a total of 201 patients who were admitted to the heart center between February and October 2019 in Beijing Chaoyang Hospital were enrolled: 175 with ACS and 26 with normal coronary arteries. The diagnosis of ACS was based on the presence of at least two of the following: dynamic ECG changes consistent with ischemia, elevated cardiac biomarkers and clinical symptoms suggestive of ischemia. All these patients underwent coronary angiography. The exclusion criteria were as follows: patients with severe valvular heart disease, acute or chronic infection, autoimmune disease, chronic renal failure (eGFR < 15 mL/min), hepatic failure, cerebrovascular disease, malignant tumors, pulmonary embolism or mental illness.

2.2 Clinical characteristics

Clinical characteristics, including age, gender, history of smoking, medical history and medication, were obtained from all subjects on admission. All subjects took at least 300 mg aspirin and 300 to 600 mg clopidogrel before coronary angiography. Electrocardiography, echocardiography, serum lipid levels, and indexes of liver and kidney functions were also recorded.

2.3 Blood assessment

Peripheral venous blood was drawn from all subjects in the early morning after hospitalization and stored in EDTA anticoagulation vacuum tubes. Plasma samples were immediately centrifuged at 3000 g for 10 min and kept at −80°C for measurement.

The plasma ELA concentration was measured using an enzyme-linked immunosorbent assay kit (S-1508.0001, Peninsula Laboratories International, San Carlos, CA) following the protocol. The kit used a double-antibody sandwich enzyme-linked immunosorbent assay to determine the level of ELA in the samples.

Serum triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), lipoprotein a [LP(a)], and indexes of liver and renal function were measured using Siemens-ADVIA 2400 system. Serum cTnI levels were detected by chemiluminescence immunoassay. CK-MB and BNP were measured by chemiluminescence assay.

2.4 Statistical analysis

Continuous variables are expressed as the mean ± SD (normal distribution) or median (quartile), and categorical variables are expressed as the frequency or as a percentage. Continuous variables were analyzed using Student’s t-test in normally distributed data and the Mann-Whitney test in nonnormally distributed data. Categorical variables were analyzed using the Chi-square test or Fisher’s exact test. P values less than 0.05 (two-sided) were considered statistically significant. All statistical analyses were performed with the statistical software packages R (http://www.R-project.org, The R Foundation) and EmpowerStats (http://www.empowerstats.com, X&Y Solutions, Inc., Boston, MA).

3 Results

3.1 Basic characteristics

Overall, 175 patients with ACS (age 62.9 ± 11.5 years, male 113 [64.6%]) and 26 with normal coronary arteries as a control (age 62.3 ± 11.4 years, male 11 [42.3%]) were included in the study. The clinical characteristics and laboratory values of the ACS and control groups are shown in Table 1. There was no statistical difference between the two groups in terms of age, body mass index, hypertension, or diabetes. Male gender and history of smoking were higher in the ACS group than in the control group (P < 0.05). The blood levels of white blood cells, aspartate aminotransferase and glucose were significantly higher in the ACS group than in the control group (P < 0.05). There were no significant differences in TC, LDL-C, alanine aminotransferase, indexes
Table 1. Baseline clinical and laboratory parameters of the study participants with and without acute coronary syndrome.

| Variable                  | Controls (n = 26) | ACS (n = 175) | P-value |
|---------------------------|------------------|--------------|---------|
| Age, yrs                  | 61.3 ± 11.4      | 62.9 ± 11.5  | 0.506   |
| BMI, kg/m²                | 26.07 ± 2.81     | 25.96 ± 3.21 | 0.875   |
| Sex                       |                  |              | 0.029   |
| Male                      | 11 (42.31%)      | 113 (64.57%) |         |
| Female                    | 15 (57.69%)      | 62 (35.43%)  |         |
| DM history                |                  |              | 0.700   |
| Yes                       | 17 (65.38%)      | 121 (69.14%) |         |
| No                        | 9 (34.62%)       | 54 (30.86%)  |         |
| Hypertension history      |                  |              | 0.025   |
| Yes                       | 12 (46.15%)      | 120 (68.57%) |         |
| No                        | 14 (53.85%)      | 55 (31.43%)  |         |
| Smoking history           |                  |              | 0.110   |
| Yes                       | 9 (34.62%)       | 90 (51.43%)  |         |
| No                        | 17 (65.38%)      | 85 (48.57%)  |         |
| SBP, mmHg                 | 130.88 ± 19.45   | 130.94 ± 21.71 | 0.990  |
| DBP, mmHg                 | 70.92 ± 8.74     | 74.36 ± 12.19 | 0.120  |
| HR, beats/min             | 72.96 ± 13.44    | 75.93 ± 10.47 | 0.203  |
| WBC, ×10⁹/L               | 6.87 ± 1.84      | 8.50 ± 3.20  | 0.012   |
| HGB, g/L                  | 136.20 ± 16.49   | 135.08 ± 16.26 | 0.748  |
| PLT, ×10⁹/L               | 221.04 ± 72.45   | 219.19 ± 61.09 | 0.830  |
| TC, mmol/L                | 4.43 ± 1.11      | 4.38 ± 1.08  | 0.847   |
| HDL-C, mmol/L             | 1.07 ± 0.30      | 0.98 ± 0.24  | 0.076   |
| LDL-C, mmol/L             | 2.57 ± 0.99      | 2.68 ± 0.98  | 0.606   |
| TG, mmol/L                | 1.50 ± 0.74      | 1.76 ± 1.26  | 0.319   |
| AST, U/L                  | 26.60 ± 15.99    | 59.34 ± 73.61 | 0.028  |
| ALT, U/L                  | 26.32 ± 18.26    | 29.37 ± 23.32 | 0.532  |
| UA, mmol/L                | 340.84 ± 80.46   | 357.78 ± 98.90 | 0.415  |
| SCR, µmol/L               | 63.88 ± 21.06    | 74.24 ± 44.26 | 0.252  |
| eGFR, mL/min per 1.73 m²  | 95.88 ± 18.52    | 90.54 ± 20.02 | 0.139  |
| Glucose, mmol/L           | 5.68 ± 1.81      | 6.97 ± 2.89  | 0.018   |
| CKMBmax, U/L              | 2.22 ± 3.41      | 36.78 ± 69.24 | P < 0.001 |
| cTNI max ng/mL            | 0.01 ± 0.02      | 37.66 ± 78.77 | P < 0.001 |
| BNPmax pg/mL              | 49.70 ± 81.85    | 325.09 ± 503.49 | P < 0.001 |

Data are presented as mean ± SD or n (%). ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; BNP: B-type natriuretic peptide; CKMB: creatine phosphokinase-MB; cTNI: cardiac troponin I; DBP: diastolic blood pressure; DM: diabetes mellitus; eGFR: estimated glomerular filtration rate; HDL-C: high density lipoprotein cholesterol; HGB: hemoglobin; HR: heart rate; LDL-C: low density lipoprotein cholesterol; PLT: platelet; SBP: systolic blood pressure; SCR: serum creatinine; TC: total cholesterol; TG: triglyceride; UA: uric acid; WBC: white blood cell.

of renal function, uric acid or other clinical characteristics between the two groups.

3.2 Comparison of ELA in the ACS and control groups

We first performed a cross-sectional comparison of the ELA levels between the ACS patients and the controls without coronary lesions. We observed that the plasma ELA levels were significantly higher in the ACS patients than in the controls (95.04 ± 18.66 vs. 71.90 ± 8.93, P < 0.01, Figure 1).

3.3 Relationship between the ELA levels and the extent of coronary lesions in the ACS group

There were no significant differences in plasma ELA among the one-, two-, and three-vessel disease groups (Figure 2). Subgroup analysis showed that ELA levels were not affected by the number of diseased vessels.

A threshold, nonlinear association between ELA and the Syntax score was found (P < 0.001) in a generalized additive model (GAM). The solid line represents the smooth curve fit between variables. Dotted lines represent the 95% confidence interval from the fit. All were adjusted for age,
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Figure 3. The relationship between ELA and SYNTAX score. A nonlinear relationship between them was detected after adjusting for age, sex, BMI, SBP and DBP. Solid red line represents the smooth curve fit between variables. Blue bands represent the 95% confidence interval from the fit. BMI: body mass index; DBP: diastolic blood pressure; ELA: Elabela; SBP: systolic pressure.

Table 2. The results of two-piecewise linear regression model.

| Inflection point of ELA | Effect size (β) | 95% CI       | P-value |
|-------------------------|----------------|-------------|---------|
| < 63.47                 | -0.33          | -0.96 to 0.31 | 0.313   |
| ≥ 63.47                 | 0.23           | 0.10 to 0.35  | < 0.001 |
| < 85.49                 | 0.71           | 0.24 to 1.17  | 0.003   |
| ≥ 85.49                 | 0.09           | -0.09 to 0.27 | 0.351   |

Effect: Syntax score; cause: ELA; adjusted: age, gender, BMI, SBP, DBP. BMI: body mass index; DBP: diastolic blood pressure; ELA: Elabela; SBP: systolic pressure.

gender, BMI, SBP and DBP. As shown in Figure 3, plasma ELA levels were positively correlated with the Syntax I score when the ELA levels ranged from 63.47 to 85.49 ng/mL (Figure 3 and Table 2). There was no significant association between the plasma ELA levels and the extent of coronary artery stenosis when the ELA levels were less than 63.47 ng/mL or higher than 85.49 ng/mL.

4 Discussion

This is an exploratory study that aimed to evaluate plasma ELA levels in ACS patients and controls without coronary lesions. We found that plasma ELA levels were higher in the patients with ACS than in the controls without coronary lesions. There was no significant correlation between plasma ELA and the number of diseased coronary arteries. However, a nonlinear relationship between ELA and Syntax I scores was detected.

To the best of our knowledge, this is the first study to explore the relationship between plasma ELA levels and the severity of coronary artery stenosis in ACS patients. We found that when plasma ELA levels ranged from 63.75 ng/mL to 85.49 ng/mL, the Syntax I score increased with increasing plasma ELA, which indicating that the higher plasma levels of ELA were significantly associated with the severity of coronary artery stenosis.

There is some evidence showing that ELA plays an important role in the homeostasis of the cardiovascular system. ELA is widely expressed in many tissues, including the heart, large conduit vessels and plasma, particularly in endothelial cells. ELA is found in plasma, and its expression is highest in embryonic heart tissue, after which it declines gradually. ELA is mainly detected in fibroblasts and endothelial cells in the heart and is essential for normal development of the heart tissue. ELA has been proven to have vasodilatory and inotropic effects. ELA induced the relaxation of the aorta through the activation of the APJ receptor in mice independent of NO. Patients with hypertension and preeclamptic patients had lower plasma ELA levels than controls. ELA expression was also decreased in patients with pulmonary artery hypertension (PAH) and rodent models of PAH. Exogenous ELA infusion significantly lowered blood pressure in high salt-loaded Dahl salt-sensitive (SS) rats. Animal studies revealed that ELA was upregulated in post-infarction cardiac remodeling and was correlated with left ventricular ejection fraction (LVEF). Treatment of Fc-ELA-21 fusion protein significantly mitigated heart dysfunction in MI rats by increasing angiogenesis and promoting cardiomyocyte proliferation. Clinical studies showed that ELA levels were significantly increased in patients with anterior and inferior ST segment elevation myocardial infarction and positively correlated with troponin I and NT-proBNP. Our study found that the proportion of patients with hypertension in the ACS group was higher than that in the control group, but the level of ELA in the ACS group was higher than that in the control group. This finding indicated that the increase in ELA levels in the ACS group was explicit and could offset the decrease in ELA levels caused by hypertension. The increase in ELA levels in our study may be related to atherosclerosis or ACS. Further studies are needed to assess the ELA and APJ receptor expression of vascular tissues and provide insights into the mechanisms of disease process of CHD and ACS.

Despite the protective effect of ELA on the cardiovascular system, the role of ELA in the pathogenesis of atherosclerosis is still unclear. Apelin and ELA are ligands of the APJ receptor. The apelinergic systems have regulatory roles in the cardiovascular system. Some studies have shown an atheroprotective effect of apelin. In an ApoE-deficient atherosclerosis model (ApoE-KO), apelin treatment mitigated...
native and AngII-accelerated atherosclerosis by promoting NO production. Human studies found that the apelin levels in patients with coronary heart disease were lower than those in controls. Our study found that plasma ELA levels were significantly higher in the patients with ACS than in the controls, which indicates that ELA may have different effects and mechanisms than apelin. There was no significant correlation between plasma ELA and the number of diseased coronary arteries. However, a nonlinear relationship between ELA and Syntax I scores was detected, which suggests that ELA levels may reflect the vulnerability of atheromatous plaques in ACS when ELA levels range from 63.47 to 85.49 ng/mL.

Atherosclerosis and related diseases are the leading causes of death. Strategies that can reduce the atherosclerotic burden and improve the stability of vulnerable plaques are required to decrease cardiovascular events. ELA is increased in ACS patients and positively correlated with the severity of coronary artery lesions. It may be a new biomarker of atherosclerosis and ACS, providing a promising future approach for the clinical assessment of patients with ACS. However, there are some limitations. First, this was a prospective cross-sectional study based on a single-center experience with a relatively small number of patients, which could result in selection bias. Further large-scale and longitudinal studies that include healthy subjects would help to clarify the relationship between ELA levels and CAD or ACS. Second, there was not enough evidence to determine the association of the level of ELA and different stages of progression of coronary heart diseases. Third, some drugs taken by ACS patients like statin, renin-angiotension system inhibitor may influence plasma ELA levels. However, in this study, we did not include sufficient data on these drugs. Finally, the causal relationship between plasma ELA levels and ACS remains unclear in this study.

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