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GRK 2 level in peripheral blood lymphocytes of elderly patients with acute myocardial infarction

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Abstract

Objective To investigate the G protein-coupled receptor kinase 2 (GRK 2) level in peripheral blood lymphocytes with cardiac function in elderly patients with acute myocardial infarction. **Methods** This study enrolled 40 patients with acute ST-segment elevation myocardial infarction (STEMI) and 40 patients with unstable angina. All patients were 65 years or older. Cardiac function was evaluated by echocardiography, and the GRK 2 level in peripheral blood lymphocytes was measured. Patients with STEMI were followed up for 2 years. **Results** The GRK 2 level in peripheral blood lymphocytes was significantly higher in patients with STEMI than in patients with unstable angina, and was negatively correlated with left ventricular ejection fraction, cardiac output, stroke volume, and left ventricular fractional shortening. The GRK 2 level was significantly elevated in some patients with acute STEMI and poor cardiac function. **Conclusions** Increased GRK 2 level in patients with acute STEMI may contribute to poor myocardial systolic function and myocardial remodeling. Measurement of the GRK 2 level in peripheral blood lymphocytes may assist in the evaluation of cardiac function and myocardial remodeling in elderly patients with acute STEMI.

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Keywords: ST-segment elevation myocardial infarction; Heart function; G protein-coupled receptor kinase 2

1 Introduction

The incidence of congestive heart failure has been increasing significantly. Ischemic heart disease is the leading cause of heart failure, and acute myocardial infarction further increases the risk of heart failure.[1] The severity of acute myocardial infarction can be stratified using clinical parameters. With recent developments in molecular biology, a variety of biological markers have also been used to assess the severity of myocardial infarction and heart failure.[2,3] However, there are currently no biological markers available to accurately predict the development of heart failure after acute myocardial infarction, and provide guidance for appropriate treatment. G protein-coupled receptor kinase 2 (GRK 2) is a GRK isozyme expressed in the heart. Chronic heart failure is characterized by β-adrenergic receptor desensitization and down-regulation induced by GRK 2 overexpression and continuous activation, resulting in systolic dysfunction and loss of beta-adrenergic receptor-dependent myocardial contractile reserve.[4–6] The myocardial GRK 2 level can reflect the degree of myocardial damage, and associated myocardiocyte apoptosis and myocardial remodeling.[7–9] Transgene-induced overexpression of βARKct in animals with heart failure significantly inhibited elevation of the GRK 2 level in left ventricular myocardiocytes, thereby improving beta-adrenergic receptor signaling, left ventricular function, and left ventricular remodeling.[10–12] The myocardial GRK 2 level is positively correlated with the GRK 2 level in peripheral blood lymphocytes, and measurement of the GRK 2 level in peripheral blood lymphocytes can therefore give an indication of the GRK 2 level in the myocardium, without requiring sampling of myocardial tissue.[13,14] The present study measured GRK 2 levels in peripheral blood lymphocytes in patients with acute ST-segment elevation myocardial infarction (STEMI), and assessed left ventricular function using echocardiography, to investigate the effects of myocardial function and remodeling on GRK 2 level in peripheral blood lymphocytes. This study aimed to provide a new index of heart failure after myocardial infarction, to assist therapeutic choices in elderly patients with acute myocardial infarction.

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2 Methods

2.1 Patients

This study included 40 patients aged 65 years or older with acute STEMI (< 24 h), and 40 age-matched patients with unstable angina as a control group. Acute STEMI was diagnosed by the symptom of chest pain and ST-segment elevation on electrocardiography and elevated creatine kinase-MB, and troponin I levels in serum. The clinical data of patients were recorded, including name, age, gender, time of onset, body height, body mass, blood pressure, heart rate, electrocardiography findings, and blood biochemistry analysis. Echocardiography was performed within 24 h from the onset of symptoms, and 10 mL of peripheral blood was drawn to measure GRK 2 levels. Patients with acute STEMI were followed up for 2 years. This study was approved by the Ethics Committee of Chinese General PLA Hospital. Written informed consent was obtained from all patients.

2.2 Echocardiography

Echocardiography was performed using the GE Vivid 7 color scanner (GE Healthcare, Milwaukee,Wisconsin,USA) to measure left ventricular ejection fraction, left ventricular fractional shortening, stroke volume, left ventricular cardiac output, and cardiac index. Peak early diastolic mitral inflow flow velocity (E), peak late diastolic mitral inflow velocity, and E/A were calculated.

2.3 Isolation of peripheral blood lymphocytes

Venous blood (10 mL) was collected into tubes containing ethylenediamine tetraacetic acid. The blood was diluted with phosphate-buffered saline, mixed, and placed in a centrifuge tube containing lymphocyte isolation solution (Sigma Aldrich, St. Louis, MO, USA) with a volume ratio of 1: 1: 1. The mixture was centrifuged at 2000 r/min for 30 min, and the middle layer (lymphocytes) was harvested, centrifuged, washed twice with phosphate-buffered saline, quantified, and stored at –80°C.

2.4 Measurement of the GRK 2 mRNA level in peripheral blood lymphocytes

Venous blood (10 mL) was collected and the lymphocytes were separated by centrifugation. The total RNA was extracted using Trizol reagent (Beijing B&M Biotech, Beijing, China) and reverse transcribed (45°C for 30 minutes, 99°C, 5min and 5°C 5min,) to obtain cDNA for PCR amplification of β-actin and GRK 2. The following primers were used. Human β-actin primer: upstream 5′-AGCGAGCAT CCCCCAAAGTT-3′, downstream 5′-GGGCACGAAGGC TCATCATT-3′; human GRK 2 primer: upstream 5′-GAAC ACATGCACAATCG-3′, downstream 5′-CCAGGGAGAA CCAGTC-3′. The primers were synthesized by SBS Genetech, Beijing, China. The amplification conditions were: 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s, for a total of 30 cycles. The products were separated by agarose gel electrophoresis and absorbance was measured.

2.5 Measurement of the GRK 2 Protein Level in Peripheral Blood Lymphocytes

The lymphocytes were lysed and centrifuged to isolate the proteins. Western blot analysis was performed using anti-GRK 2 and anti-actin polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Protein expression was quantified using a standard enhanced chemiluminescence kit (Amersham Biosciences, Piscataway, NJ, USA). The GRK 2 levels in lymphocytes were corrected using actin expression.

2.6 Statistical analysis

All parameters are expressed as mean ± SD; SPSS18.0 statistical software is adopted for data analysis; comparisons between groups use chi-square test. $P < 0.05$ presents that differences are statistically significant.

3 Results

3.1 Baseline data

There were no significant differences in baseline characteristics between patients with acute STEMI and patients with unstable angina, except that the heart rate was significantly higher in patients with acute STEMI (Table 1). Left ventricular ejection fraction, left ventricular fractional shortening, stroke volume, and cardiac output were significantly lower in patients with STEMI than in patients with unstable angina (Table 2).

Table 1. Baseline data of patients.

	Unstable angina patients (n = 40)	STEMI patients (n = 40)
Men/women	36/4	37/3
Age (yrs)	76.43 ± 3.49	75.74 ± 7.64
Body mass (kg)	69.98 ± 10.99	65.24 ± 10.52
Body mass index (kg/m^2)	24.21 ± 3.12	22.80 ± 3.09
BSA (m^2)	1.78 ± 0.17	1.71 ± 0.16
SBP (mmHg)	136.92 ± 19.07	127.67 ± 17.33
DBP (mmHg)	17.33 ± 9.22	67.30 ± 9.86
Heart rate (beats/min)	69.35 ± 9.46	84.67 ± 15.94$^{\#}$

$^{\#}P < 0.01$. BSA: body surface area; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table 2. Echocardiogram parameters.

	STEMI patients (n = 40)	Unstable angina patients (n = 40)
EF (%)	46.53 ± 2.86	56.65 ± 3.95#
Shortening fraction (%)	29.63 ± 2.69	31.97 ± 2.88*
Stroke volume (mL)	42.17 ± 3.16	65.62 ± 5.52*
CO (L/min)	3.21 ± 0.22	4.32 ± 0.39*
CI (L/min/m^2)	1.89 ± 0.13	2.27 ± 0.15
E (cm/s)	68.49 ± 15.99	77.04 ± 22.64
A (cm/s)	101.43 ± 21.79	98.27 ± 17.50
E/A ratio	0.79 ± 0.31	0.72 ± 23

*P < 0.05; #P < 0.01. CI: cardiac index; CO: cardiac output; EF: ejection fraction; E/A ratio: early (E) to late (A) ventricular filling velocities.

3.2 GRK 2 level in peripheral blood lymphocytes of elderly patients with STEMI

RNA and protein were extracted from the peripheral blood lymphocytes of patients with STEMI and unstable angina to measure the GRK 2 levels. The lymphocyte GRK 2 mRNA and protein levels were significantly higher in patients with acute STMEI than in patients with unstable angina (P < 0.01; Figure 1).

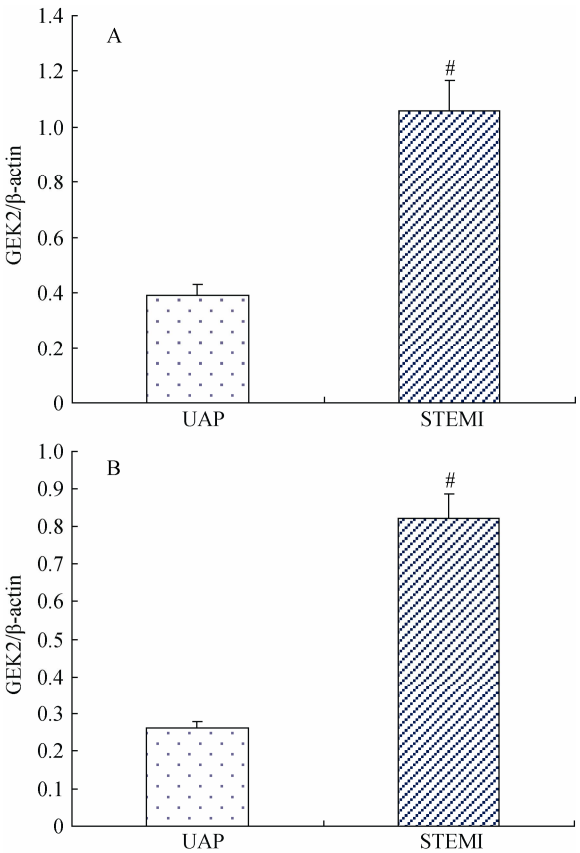

Figure 1. The GRK 2 levels in peripheral blood lymphocytes in the two groups. (A): GRK 2 mRNA levels; (B): GRK 2 protein levels. #P < 0.01 *vs*. patients with unstable angina. UAP: unstable angina pectoris; STEMI: ST-segment elevation myocardial infarction.

3.3 Left ventricular function and the GRK 2 level in peripheral blood lymphocytes of patients with STEMI after two years

After two years, 40 patients with unstable angina had normal cardiac function, and the GRK 2 level in peripheral blood lymphocytes was not significantly changed (data omitted). Six of the 40 STEMI patients still had poor cardiac function (EF < 50%) and enlargement of the left ventricle, the others had got normal cardiac function. The GRK 2 level in peripheral blood lymphocytes was significantly higher in these six patients than in the 34 patients with normal cardiac function (Figure 2).

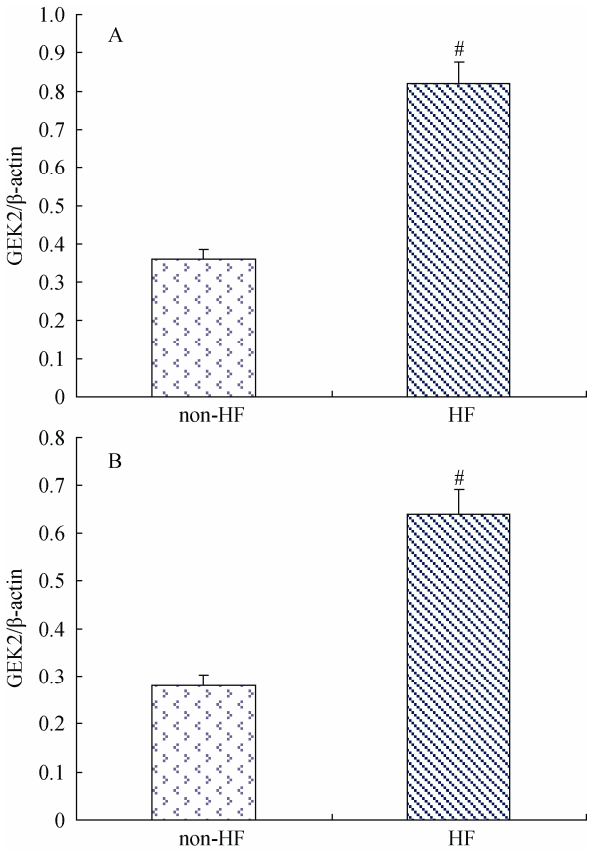

Figure 2. Left ventricular function and the GRK 2 level in peripheral blood lymphocytes of patients with STEMI after 2 years. (A): GRK 2 mRNA levels; (B): GRK 2 protein levels. #P < 0.01 comparison between patients with poor cardiac function and those with normal cardiac function. HF: heart failure. STEMI: ST-segment elevation myocardial infarction.

4 Discussion

This is the first study to assess cardiac function and GRK 2 levels in elderly patients with acute STEMI. The GRK 2 level in peripheral blood lymphocytes was significantly

higher in patients with acute STEMI than in patients with unstable angina. After two years, the GRK 2 level in the peripheral blood lymphocytes was higher in STEMI patients with poor cardiac function than in patients with normal cardiac function.

Our results were similar to those of a previous study of 32 patients with acute STEMI, but there were also some notable differences.[15] In this study, the GRK 2 level in peripheral blood lymphocytes was elevated in patients with low left ventricular ejection fraction, possibly because elderly patients have less myocardial contractile reserve than younger patients. In addition, there was no significant difference in E/A (early (E) to late (A) ventricular filling velocities) ratio between the two groups. This may be because the elderly patients in our study had a prolonged history of coronary artery disease, and had poor cardiac diastolic function.

The baseline heart rate was higher in patients with STEMI than in patients with unstable angina. This may be because of a combination of poor cardiac function and increased adrenergic stimulation in our elderly patients. This is also a possible cause of the elevated GRK 2 level in peripheral blood lymphocytes of patients with STEMI.[16,17]

Animal studies found that GRK 2 had negative effects on the β-receptor signaling system. GRK 2 gene knockout or transgenic techniques can inhibit GRK 2 activity, thereby reducing the progression of myocardial infarction to heart failure, and restoring cardiac function.[18–20] Our previous studies found that GRK 2 level was elevated in elderly patients with chronic heart failure, and that metoprolol could lower the GRK 2 level in these patients.[21,22] This study showed that the GRK 2 level in peripheral blood lymphocytes was significantly lower in patients with STEMI whose cardiac function had improved significantly after two years than in those with poor cardiac function and myocardial remodeling after two years. This indicates that the GRK 2 level can reflect cardiac function and provide information about myocardial remodeling. Measurement of the GRK 2 level in peripheral blood lymphocytes can therefore assist in the understanding of cardiac function and myocardial remodeling in patients with myocardial infarction. Because GRK 2 level in peripheral blood lymphocytes indirectly reflect state of beta-adrenergic receptor in myocardium, measuring GRK 2 levels would provide reference for β-receptor blockade administrated in patients with acute myocardial infarction and heart failure, and could help to make therapeutic choices in patients with acute myocardial infarction by measuring GRK 2 levels. However, this study had a small sample size. Further studies with larger sample sizes are needed to verify the results.

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