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Expression of profilin-1 in endothelial cells of rats with acute myocardial infarction

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Abstract. – OBJECTIVE: To analyze the expression of profilin-1 in endothelial cells of rats with acute myocardial infarction.

MATERIALS AND METHODS: Twenty adult male Wistar rats were randomly divided into the myocardial infarction (model) group (n=10) and sham-operation (control) group (n=10). The expression of profilin-1 and phosphorylated extracellular signal kinase (pERK1/2) in aortic endothelial cells, indexes of endothelial injury [levels of endothelial microparticles (EMPs) and nitric oxide (NO)], indexes of myocardial injury [cardiac troponin T (cTnT) and creatine kinase-MB (CK-MB)], and mRNA levels of myocardial apoptotic factors (P53, Fas, Bax, and Bcl-2) in rats between the two groups were compared.

RESULTS: The expression of profilin-1 and pERK1/2 in aortic endothelial cells of rats in the model group was higher than in the control group ($p<0.05$), the levels of EMPs were increased, and NO levels were lower ($p<0.05$); cTnT and CK-MB in myocardial tissue, and mRNA of pro-apoptotic factors (P53, Fas, and Bax) were increased, whereas Bcl-2 mRNA was decreased ($p<0.05$). The protein expression of profilin-1 and pERK1 was positively correlated with the levels of cTnT, CK-MB, EMP, P53, Fas, and Bax, and negatively correlated with the levels of NO and Bcl-2 ($p<0.05$).

CONCLUSIONS: The high expression of profilin-1 is an important mechanism of acute myocardial infarction, and is expected to become a new target for the treatment of myocardial infarction.

Key Words:
Acute myocardial infarction, Profilin-1, Endothelial cells.

Introduction

The occurrence of acute myocardial infarction (AMI) is directly related to coronary endothelial dysfunction and is characterized by the imbalance of vasoconstrictive and vasodilatory factors produced by endothelial cells[1,2]. The expression of profilin-1 has been shown to be upregulated in endothelial cells in patients with hypertension, resulting in abnormal activation of relevant signaling pathways. Inhibition of profilin-1 expression can lower blood pressure and reduce systemic oxidative stress[3]. Hypertension is an important factor in the occurrence of coronary endothelial injury, atheromatous plaque instability, and cardiovascular events. Therefore, it is speculated that profilin-1 plays an important role in AMI. Profilin-1 can promote the proliferation and migration of vascular endothelium, and inhibition of profilin-1 can reduce endothelial damage induced by the basement membrane[4,5]. To determine the specific role of profilin-1 in AMI, we established a model of AMI to analyze its correlation with myocardial injury and myocardial apoptosis, to provide a basis for further study.

Materials and Methods

Animals and Experimental Instruments

Twenty healthy specific pathogen free (SPF)-level male Wistar rats weighing 200 ± 10 g were provided by the Animal Center of the Affiliated Medical College of Beijing Anzhen Hospital. Rats underwent adaptive breeding for 1 week. Cardiac troponin T (cTnT) and creatine kinase-MB (CK-MB) kits were from Shanghai Rongchuang Biological Technology Co., Ltd., and a fully-automatic biochemical analyzer (AU5800 series) was from Beckman Coulter (Fullerton, CA, USA).

Establishment of the Rat Model of AMI and Experimental Grouping

Twenty rats were randomly divided into the model group (n=10) and the control group (n=10). They received intraperitoneal injections of 3% pentobarbital at a dose of 60 mg/kg. The neck and chest hair was shaved off after 3 min, and a 1-cm-long median incision was made in the skin after disinfection. The

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organs were exposed and connected to the breathing machine after insertion of a tracheal cannula (tidal volume of 7 ml/kg, frequency of 20-30 times/min, inspiratory/expiratory ratio of 1:1). The skin of the chest was disinfected, the chest muscle was separated by blunt dissection, and the ribs were exposed. Next, 3-4 ribs were cut off, the chest was expanded, and the heart was exposed. The rats in the control group underwent sham surgery. After the heart was exposed, the muscle and skin were sutured layer-by-layer, and the air in the chest was removed. The breathing machine was removed after rats woke up, and they were given access to food in their cage.

For rats in the model group, the pericardium was removed with ophthalmic forceps. A No.0 line was inserted between the left auricle and arterial cone, and ligated. If ECG showed typical ischemic imaging, the ligation in the left anterior descending artery was considered successful, and the model of AMI was established. The muscle and skin of rats were sutured layer-by-layer, and the air in the chest was removed. The breathing machine was removed after rats woke up, and they were given access to food in their cage.

Observational Indexes and Test Methods

At 24 h after the establishment of the model, rats in the model group and control group were sacrificed. The aortic tissues were harvested, homogenized, and stored at −80°C until use.

Measurement of Profilin-1 and pERK1/2

Western blot was used to measure the levels of profilin-1 and phosphorylated extracellular signal kinase protein (pERK1/2) in rat aortic tissue samples. Bradford assay was used to measure the protein content in samples. A total of 50 μg protein was separated by electrophoresis in 12% polypropylene amide gels, and blocked in 5% milk for 1 h at room temperature. Membranes were incubated overnight with an anti-profilin-1 primary antibody or anti-pERK1/2 primary antibody (Thermo Fisher Scientific, Waltham, MA, USA). After washing, membranes were incubated with horseradish peroxidase-labeled second antibody at room temperature for 1 h, and bands were exposed. β-actin served as the internal reference, and semi-quantitative analysis was conducted for the profilin-1 and pERK1/2 bands.

Detection of Indexes of Endothelial Injury, Myocardial Injury, and Apoptosis

Flow cytometry was used to measure the levels of endothelial microparticles (EMPs) in homogenates, spectrophotometry was used to measure the nitric oxide (NO) level, and the fully-automatic biochemical analyzer was used to measure the levels of cTnT and CK-MB.

Total RNA was extracted from myocardial homogenates. Total RNA was washed with 75% ethyl alcohol and dissolved in DEPC water. Next, adequate amounts of RNA were reverse transcribed into cDNA using a reverse transcription kit (Fermentas, Wilmington, NC, USA, Item No. K1622). A quantitative fluorescence PCR kit (Shanghai Kemin Biological Technology Co., Ltd., China) was used for amplification. The PCR amplification curve was obtained on a computer. The levels of P53, Fas, Bax, and Bcl-2 mRNA in myocardial tissue of rats in the control group were set as 100, and the relative mRNA levels of these target genes in rats of the model group were calculated.

Statistical Analysis

SPSS23.0 software (SPSS Inc., Chicago, IL, USA) was used for data input and analysis. Quantitative data are presented as mean ± standard deviation, and t-test was used for comparisons. After normality test, a Pearson test was used to detect the correlation of quantitative data. $p<0.05$ was considered statistically significant.

Results

Expression of Profilin-1 and pERK1/2

The expression of profilin-1 and pERK1/2 in aortic endothelial cells from rats of the model group was higher than in rats of the control group ($p<0.05$) (Figure 1).

Comparison of Indexes of Endothelial Injury

The levels of EMPs in aortic tissue from rats of the model group were increased, whereas the levels of NO were decreased ($p<0.05$) (Table I).

Comparison of Indexes of Myocardial Injury

The levels of cTnT and CK-MB in myocardial tissue from rats of the model group were higher than those of the control group ($p<0.05$) (Table II).

Comparison of Indexes of Myocardial Apoptosis

The mRNA expression of pro-apoptotic factors such as P53, Fas, and Bax, in rats of the model group, was higher than in rats of the control

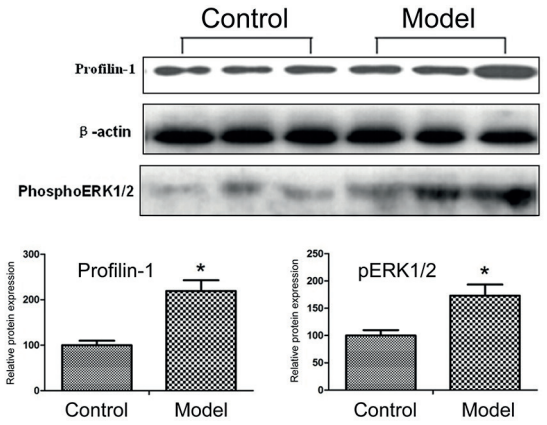

Figure 1. Expression of profilin-1 and pERK1/2.

Table I. Comparison of indexes of endothelial injury.

Group	EMP (/50 μl)	NO (μmol/l)
Model group	33.28±4.14	45.38±5.81
Control group	10.76±1.32	69.27±7.14
t	7.293	7.682
p	0.008	0.003

Table II. Comparison of indexes of myocardial injury.

Group	cTnT (ng/l)	CK-MB (U/l)
Model group	23.17±4.51	164.17±40.33
Control group	0.55±0.17	33.28±8.29
t	56.293	62.273
p	0.000	0.000

group. In contrast, the mRNA expression of the anti-apoptotic factor, Bcl-2, in the model group was lower than in the control group ($p<0.05$) (Table III).

Correlation between the Protein Expression of Profilin-1 and pERK1/2 and other Indexes

Pearson test showed that the protein expression of profilin-1 and pERK1/2 in endothelial cells was positively correlated with the levels of cTnT, CK-MB, EMPs, P53, Fas, and Bax, and negatively correlated with the levels of NO and Bcl-2 ($p<0.05$) (Table IV).

Discussion

Many studies have shown that patients with AMI suffer from vascular endothelial dysfunction. Vascular endothelial dysfunction is the initial factor causing atherosclerosis and development of coronary heart disease[6]. According to some studies[7], Atorvastatin can protect vascular endothelial function, so that myocardial apoptosis in AMI is inhibited. Ding et al[4] suggested that there is a direct relationship between the level of serum profilin-1 and the degree of coronary artery stenosis in patients with coronary heart disease. Romeo et al[5] confirmed that profilin-1 plays an important role in mediating vascular endothelial injury. profilin-1 belongs to the family of actin-binding proteins, and is involved in regulating the dynamic levels of actin. Profilin-1 interacts with many proteins, and plays a role in the regulation of signal transduction in the cardiovascular system. Profilin-1 acts via the PIP2, MAPK, JNK, and other signaling pathways. Changes in function of the pERK1/2 signaling pathway directly affect the role of profilin-1. In this study, profilin-1 and pERK1/2 were shown to be highly expressed in the aorta of rats with AMI. It was shown *in vitro* that various mediators of endothelial cell injury can increase the expression of profilin-1 in endothelial cells, leading to reorganization of the endothelial cytoskeleton[8]. This indicates that high expression of profilin-1 and pERK1/2 in aortic endothelial cells can lead to endothelial dysfunction and participation in the development of AMI. The levels of EMPs in rats with AMI were increased, whereas the levels of NO were decreased. EMPs are membrane vesicles released following endothelial cell activation and apoptosis. They directly reflect endothelial cell dysfunction and

Table III. Comparison of indexes of myocardial apoptosis.

Group	P53	Fas	Bax	Bcl-2
Model group	175.49±20.17	173.26±19.58	213.27±24.51	58.39±6.11
Control group	100±9.21	100±10.83	100±9.63	100±10.17
t	8.293	8.271	10.283	9.283
p	0.000	0.000	0.000	0.000

Table IV. Correlation between the protein expression of Profilin-1 and pERK1/2 and other indexes.

	Profilin-1		pERK1/2	
Index	Coefficient of determination r	p	Coefficient of determination r	p
cTnT	0.373	0.031	0.312	0.020
CK-MB	0.402	0.021	0.395	0.022
EMP	0.493	0.021	0.373	0.027
NO	-0.312	0.020	-0.304	0.021
P53	0.484	0.025	0.316	0.019
Fas	0.435	0.015	0.358	0.029
Bax	0.494	0.023	0.324	0.016
Bcl-2	-0.323	0.017	-0.384	0.028

participate in the pathophysiology of diseases of the circulatory system. The level of NO correlates with vasodilatory function, and decreased levels of NO can cause dysfunction of vasodilatation and continuous vasoconstriction[9-12].

High level of myocardial apoptosis after AMI is the most direct cause of irreversible damage to myocardial cells. Four apoptosis-related genes, P53, Fas, Bax, and Bcl-2, are well characterized. The overexpression of P53 can induce myocardial apoptosis. However, myocardial apoptosis still occurs in mice lacking P53, indicating that there are pro-apoptotic mechanisms other than P53. In apoptosis induced by myocardial cell hypoxia, the expression of the Fas gene can be upregulated. Therefore, Fas is a pro-apoptotic gene. The pro-apoptotic function of Bax is inhibition of Bcl-2, and the expression of Bcl-2 can reduce apoptosis of myocardial tissue induced by ischemia-reperfusion. In this study, we found that P53, Fas, and Bax mRNA in myocardial tissue from rats with myocardial infarction was increased, while Bcl-2 mRNA was decreased, indicating that imbalances in pro-apoptotic and anti-apoptotic genes in myocardial tissue are involved in the pathogenesis of AMI[13-15].

Correlation analyses showed that the protein expression of profilin-1 and pERK1/2 was positively correlated with the levels of cTnT, CK-MB, EMPs, P53, Fas, and Bax, and negatively correlated with the levels of NO and Bcl-2.

Conclusions

The high expression of profilin-1 is an important mechanism of AMI. Profilin-1 is expected to become a new target for the treatment of myocardial infarction.

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Conflict of interest

The authors declare no conflicts of interest.

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