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Significance of serum 8-*iso*-prostaglandin $F_{2\alpha}$ level in acute myocardial ischemia and the therapeutic effect of N-acetylcysteine in rats

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Abstract: **Objective** 8-*iso*-8-iso-prostaglandin $F_{2\alpha}$ (8-*iso*-$PGF_{2\alpha}$) is an index that can sensitively and specifically reflect peroxidation caused by increased free radicals after reperfusion. N-acetlycysteine (NAC) acts as an effective scavenger for free radical cleaning in the body. This study aimed to analyze the correlation of 8-*iso*-prostaglandin $F_{2\alpha}$ level between serum and myocardial tissue, and to investigate the therapeutic effect of NAC and the significance of serum 8-*iso*-$PGF_{2\alpha}$ level in rats with acute myocardial ischemia. **Methods** Forty-five male manhood Wistar rats were divided into three groups (15 rats in each group): Ischemia, NAC, and Control groups. Rats in the NAC group were given gastric lavage with NAC (0.1g/kg per day) for three weeks. Two weeks later the Ischemia and NAC groups were subjected to acute myocardial ischemia induced by intraperitoneal injection of pituitrin (20 U/kg), and the elevation of the ST segment in the ECG was used as the index of myocardial ischemia. The Control rats were injected intraperitoneally with normal saline. The 8-*iso*-$PGF_{2\alpha}$ contents in serum and myocardial tissue were determined by ELISA. **Results** Compared with the Control group, the 8-*iso*-$PGF_{2\alpha}$ contents in serum and myocardial tissue of the Ischemia group were significantly higher (60.4 ± 13.7 pg/mL vs 187.4 ± 45.8 pg/mL and 88.6 ± 16.9 pg/mL vs 259.3 ± 47.5 pg/g, both $P < 0.01$) In the NAC group, 8-*iso*-$PGF_{2\alpha}$ concentrations of serum and myocardial tissue were 88.2 ± 16.4 pg/mL and 109.4 ± 24.7 pg/g respectively, lower than those in the Ischemia group ($P < 0.01$). The 8-*iso*-$PGF_{2\alpha}$ levels of serum and myocardial tissue were positively correlated ($r = 0.865$, $P < 0.01$). In comparison with the controls, ST segments of ECG in rats with myocardial ischemia elevated and the peak of ST segments occurred 45 minutes after myocardial ischemia (0.34 ± 0.05 mV) ($P < 0.01$). Pre-treatment with NAC improved myocardial ischemia (the elevation of ST segment was only 0.18 ± 0.05 mV). **Conclusions** The level of 8-*iso*-$PGF_{2\alpha}$ increased in both serum and myocardial tissues of rats with acute myocardial ischemia. The serum level of 8-*iso*-$PGF_{2\alpha}$ can be used to evaluate the severity of myocardial ischemia. NAC reduces the free radical damage in myocardial tissues, and therefore, it may improve the amelioration of the heart during myocardial ischemia. [**Chin J Contemp Pediatr**, **2005**, **7**(**1**):**8-11**]

Key words: Myocardial ischemia; Oxygen free radical; 8-*iso*-prostaglandin $F_{2\alpha}$; N-acetlycysteine; Rat

心肌缺血大鼠血浆 8-异前列腺素 $F_{2\alpha}$ 水平及 N -乙酰半胱氨酸干预作用

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[**摘　要**] **目的**　8-异前列腺素 $F_{2\alpha}$ (8-*iso*-$PGF_{2\alpha}$) 是一种敏感、特异性反映缺血-再灌注后氧自由基增加的生化指标。抗氧化剂 N-乙酰半胱氨酸(NAC)具有清除氧自由基的作用。本研究旨在分析实验性心肌缺血大鼠血浆与心肌 8-*iso*-$PGF_{2\alpha}$ 相关性以及 NAC 的治疗效果，探讨血浆 8-*iso*-$PGF_{2\alpha}$ 反映心肌氧自由基损伤程度的可能性和 NAC 的干预效果。**方法**　45 只雄性成年 Wistar 大鼠随机分为 3 组(每组 15 只)：对照组、缺血组和 NAC 组。缺血组和 NAC 组腹腔注射垂体后叶素(20 U/kg)制成大鼠急性心肌缺血模型，以心电图上 ST 段的抬高作为心肌缺血的指标。对照组仅腹腔注射生理盐水。NAC 组缺血前 2 周开始用 NAC(每日 0.1g/kg)灌胃，共 3 周。应用 ELISA 方法测定各组大鼠血浆及心肌组织 8-*iso*-$PGF_{2\alpha}$ 含量。**结果**　缺血组大鼠的血浆和心肌组织含量分别为(187.1 ± 45.8) pg/mL 和(259.3 ± 47.5) pg/g，明显高于正常对照组(60.4 ± 13.7) pg/mL 和(88.6 ± 16.9) pg/g

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($P < 0.01$)；NAC 组的血浆和心肌组织 8-*iso*-$PGF_{2\alpha}$含量为(88.2 ± 16.4) pg/mL 和(109.4 ± 24.7) pg/g 明显低于缺血组($P < 0.01$)。血浆与心肌 8-*iso*-$PGF_{2\alpha}$水平相关($r = 0.856$, $P < 0.01$)。与正常组比较,缺血组的心电图 ST 段明显抬高(心肌缺血 45min 时抬高最为明显,达 0.34 ± 0.05 mV)($P < 0.05$)；NAC 组大鼠心肌缺血明显改善(心肌缺血 45min 时心电图 ST 段仅抬高 0.18 ± 0.05 mV)。**结论** 急性心肌缺血时存在氧自由基损伤,8-*iso*-$PGF_{2\alpha}$含量产生增加。血浆8-*iso*-$PGF_{2\alpha}$可反映心肌缺血程度。NAC 具有消除氧自由基作用,可治疗心肌缺血。

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[关 键 词] 心肌缺血;氧自由基;8-异前列腺素 $F_{2\alpha}$;乙酰半胱氨酸;大鼠
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Studies have shown that free radicals generated by reperfusion after myocardial ischemia can induce myocardial damages. Anti-oxidants can eliminate free radicals and therefore protect myocardium. Recent studies have also discovered some biochemical markers that can reflect free radical levels, such as superoxide dismutase (SOD), lipid peroxidase (LPO), glutathione peroxidase (GSH-PX) and malondialdehyde (MDA) in the body. However, these parameters are significantly affected by factors *in vitro* and *in vivo*, therefore they can not correctly reflect the exact levels of free radicals generated in the body[1]. 8-*iso*-$PGF_{2\alpha}$, a non-enzymatic product of peroxidation of arachidonyl-containing phospholipids catalyzed by free radicals, can not only constrict renal, pulmonary and coronary arteries, but can also modulate platelet adhesion[1-4]. It was found that 8-*iso*-$PGF_{2\alpha}$ is a stable compound in tissues and can sensitively and specifically reflect peroxidation caused by increased free radicals after reperfusion[5].

N-acetlycysteine (NAC) is widely used as an expectorant in clinics. In fact, NAC also acts as an effective scavenger of oxygen free radicals, maintains the oxidation-reduction balance and can protect cells from oxidative damages. But it is unknown whether NAC can reduce oxidative injury in myocardial ischemia. In this research, a rats model of acute myocardial ischemia was used to analyze the correlation between the levels of 8-*iso*-$PGF_{2\alpha}$ in serum and in myocardium, and to study the possibility of 8-*iso*-$PGF_{2\alpha}$ as an indication for myocardial ischemia and to evaluate the therapeutic effects of NAC.

Materials and methods

Animals and grouping

Forty-five male manhood Wistar rats, weighing between 160 g and 180 g, were supplied by the Medical Experiment Center of Guangdong Province. They were randomly divided into three groups ($n = 15$ each): Ischemia, NAC and Control group. Rats in the Ischemia group and NAC group were subjected to acute myocardial ischemia induced by intraperitoneal injection of 20 U/kg pituitrin (Shanghai Biochemistry Pharmaceutical Ltd). The control rats were injected intraperitoneally with normal caline. Their electrocardiograms were recorded. NAC group was applied gastric lavage with NAC (0.1 g/kg per day) for 3 weeks, while the other two groups were applied gastric lavage with normal saline instead. NAC (Fulushi, 100 mg/bag, produced by Hainan Jinxiao Pharmaceutical Ltd) was dissolved in distilled water and the final concentration was 5%.

Collection of samples

All the rats were sacrificed 1 hour after injection. Then 5 mL of serum was collected. About 100 mg of ventricle tissues were removed, washed with phosphate buffer saline (PBS) and stored at - 20℃ before use. Before 8-*iso*-$PGF_{2\alpha}$ determination, the samples were thawed at 4℃ and then 10 times volume HEPES was added. The tissues were homogenized and centrifuged at 12 000 rpm for 20 minutes. The supernatants were collected and stored at - 4℃.

Determination of 8-*iso*-$PGF_{2\alpha}$

ELISA was used to determine 8-*iso*-$PGF_{2\alpha}$ in serum and myocardial tissues. The ELISA kits were purchased from the Cayman Chemical Company, USA. The samples were analyzed using Microplate analyzer Anths HT3 (AUSLAB Company, Switzerland). Inter-group and intra-group variation coefficients were 6.8% and 10.2%, respectively. The sensitivity of the test was 10 pg/mL.

Statistic analysis

The data were analyzed using SPSS 10 softeware. Differences among multiple groups were analyzed by the one-way ANOVA. An SNK-*q* test was performed to analyze the differences between two groups. Linear regression was used to analyze correlation between 8-*iso*-$PGF_{2\alpha}$ level in serum and that in myocardial tissues.

Results

Dynamic analysis of the ST segment in ECG

In the Ischemia group, ST segments elevated at 15, 30, 45 and 60 minutes post-ischemia (0.12 ± 0.02, 0.17 ± 0.04, 0.34 ± 0.05 and 0.17 ± 0.04 mV, respectively), and reached a peak at 45 minutes post-ischemia, which was significantly higher than that of the Control group (0.03 ± 0.01 mV, $P = 0.0025$). ST segments of the NAC group also elevated at 15, 30, 45 and 60 minutes post-ischemia (0.10 ± 0.02, 0.15 ± 0.02, 0.18 ± 0.05 and 0.15 ± 0.02 mV, respectively). But the elevation extent at 45 and 60 minutes post-ischemia was significantly lower than that of the Ischemia group (both $P < 0.001$).

8-*iso*-$PGF_{2\alpha}$ levels in serum and myocardial tissues

Compared with the Control group, 8-*iso*-$PGF_{2\alpha}$ levels in serum and myocardium significantly increased after acute myocardial ischemia ($P < 0.001$). After NAC pretreatment, 8-*iso*-$PGF_{2\alpha}$ levels significantly decreased, but were still higher than those of the controls ($P < 0.001$) (Table 1).

Table1 Levels of 8-*iso*-$PGF_{2\alpha}$ in serum and myocardium of various groups ($n = 15$, $\bar{x} \pm s$)

Group	Serum (ng/mL)	Myocardium (ng/g)
Control	60.4 ± 13.7	88.6 ± 16.9
Ischemia	187.1 ± 45.8[a]	259.3 ± 47.5[a]
NAC	88.2 ± 16.4[a,b]	109.4 ± 24.7[a,b]
F	78.12	123.83
P	< 0.001	< 0.001

a compared with the Control group $P < 0.01$, b compared with the Ischemia group $P < 0.01$

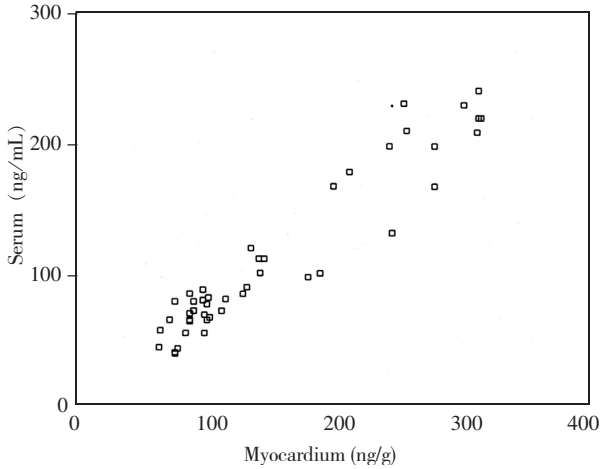

Figure 1 The correlation of serum and myocardial tissues 8-*iso*-$PGF_{2\alpha}$ contents

Correlation of 8-*iso*-$PGF_{2\alpha}$ levels between serum and myocardium

There was a linear correlation of 8-*iso*-$PGF_{2\alpha}$ levels between serum (S) and myocardium (C) ($F = 54.95$, $P = 0.003$). This can be expressed in the equation: $S = 3.361 + 0.712\ C$, with a standard partial regression coefficient of 0.712 and a correlation coefficient of 0.856.

Discussion

Ischemia can induce severe damage to myocardial cells. Subsequently, reperfusion could accelerate the death of cells due to lipid peroxidation caused by abundant free radicals[6-8].

Normally, production and elimination of free radicals in the body are maintained at a dynamic balance. Free radicals can convert into oxygen and water under the action of enzymes as SOD and GSH-PX. After myocardial ischemia, intracellular SOD decreases and free radicals accumulate immediately. Free radicals attack the double bonds of arachidonic acid in cell membranes, resulting in lipid peroxidation and the chain reactions of the free radicals[3,9]. 8-*iso*-$PGF_{2\alpha}$ is a bioactive compound that is structurally similar to prostaglandins. It is the product of peroxidation of arachidonic acid in the cell membrane. 8-*iso*-$PGF_{2\alpha}$ is stable in tissues because it is a product of non-enzymatic process. It is believed to be an ideal biomarker for the free radicals and is used to evaluate the efficacy of anti-oxidation drugs in clinics[5,9]. Studies have shown that 8-*iso*-$PGF_{2\alpha}$ in serum and in urine increased in diseases of the nervous and respiratory systems, as well as in hepatocirrhosis and diabetes mellitus. 8-*iso*-$PGF_{2\alpha}$ has been found to be positively correlated with the severity of these diseases[1,3]. 8-*iso*-$PGF_{2\alpha}$ levels rise in cardiovascular disease. In an animal model of hypercholesterolemia, 8-*iso*-$PGF_{2\alpha}$ increased and caused constriction of coronary arteries[10]. Free radicals could induce atherosclerosis, which was also highly correlated with the level of 8-*iso*-$PGF_{2\alpha}$[5,6]. In patients with heart failure, the levels of 8-*iso*-$PGF_{2\alpha}$ in serum and in urine were positively correlated with the severity of the disease[3]. Our provious studies found that the levels of 8-*iso*-$PGF_{2\alpha}$ in serum could be used to evaluate the conditions and prognosis of patients with coronary heart disease[11]. This study showed that the level of 8-*iso*-$PGF_{2\alpha}$ increased significantly in both rat serum and

myocardium. These findings were in accordance with the report of other cardiovascular diseases mentioned above. The levels of 8-*iso*-$PGF_{2\alpha}$ in serum and myocardium showed a linear relationship, which suggests that 8-*iso*-$PGF_{2\alpha}$ generated after myocardial ischemia can enter into the blood circulation, and thus, the level of 8-*iso*-$PGF_{2\alpha}$ in serum can be used to evaluate the severity of ischemia and efficiency of its therapy.

Pituitrin can cause shrinkage of coronary arteries and myocardial ischemia, and an elevated ST segment can be observed. Although elevated ST segments were observed in the NAC-pretreated rats, the change is much smaller than that in ischemia rats. It indicated that the antioxidant NAC could reduce the damage caused by reperfusion after myocardial ischemia. NAC may eliminate free radicals by the following mechanisms: 1) NAC directly acts as an anti-oxidant; 2) NAC is a small molecule, which can enter into cells easily and be converted into Cys by removal of its acetyl group. NAC could accelerate the synthesis of GSH, an important reductants, in cells. 3) NAC may upregulate biosynthesis of enzymes involved in elimination of free radicals such as SOD and GSH-PX[12-14].

In conclusion, this research found that serum level of 8-*iso*-$PGF_{2\alpha}$ can be used to estimate the damage of oxygen free radical, and the degree of myocardial injury. NAC can effectively eliminate free radicals and improve myocardial ischemia. So, it may be a promising therapy for acute myocardial ischemia.

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