Changes and Significance of NF-κB Expression in Peripheral Blood of Older Patients with Acute Coronary Syndrome

Duan Mingqin, Wang Lixia, Cao Xuanchao, Xu Xianjing

Kang Xin Comprehensive Ward, Henan Provincial People's Hospital, Zhengzhou, Henan 450003, China
E-mail: mingqinduan@sina.com

Abstract: Objective To detect the NF-κB expression of mononuclear cells in peripheral blood of older patients with acute coronary syndrome before and after drugs treatment, and discuss the relations and clinical significance among NF-κB, coronary artery lesions and drug efficacy. Method Choosing 88 older patients with acute coronary syndrome (ACS) who were verified by coronary angiography as the research group and additionally 56 older patients with normal coronary angiography as a control group, taking all patients’ blood sample from cubital veins respectively after admission and treatment for a month, determining the NF-κB expressing activity of mononuclear cells in peripheral blood by flow cytometry, and then observing the changes before and after treatment. Result There was no statistical difference in gender, age, TC and HDL-C between the two groups, but the proportion of diabetes and TC, LDL-C levels in patients of ACS group were significantly higher than those in the control group (P<0.01); levels of hs-CRP, NF-κB patients with ACS were significantly higher than those of control group (P<0.01). In ACS group levels of hs-CRP and NF-κB decreased obviously after treatment for a month (P <0.01). Conclusion A detection for levels of NF-κB expression activity not only has a certain clinical significance in pathogenetic condition and prognosis estimation, but also has a certain value on assessing the curative effect of crown expansion drugs of ACS, lipid-lowering drugs and platelet aggregation inhibitors.

Key words: acute coronary syndrome; nuclear factor-κ B; inflammatory responses

Introduction Nuclear Factor-KB (NF-κB) firstly discovered in lymphocytes by Sen and Baltimore in 1986 is a nuclear transcription factor which can combine with sequence-specificity of enhancers of immunoglobulin’s K Light-Chain Gene. NF-κB is an important modulator in most physiological and pathological processes including innate immune responses, inflammatory responses, proliferation, tumors and apoptosis, etc. Many studies suggest that the inflammation and immunity join in the genesis and development of atherosclerosis and plaque rupture, the activation of inflammatory reactions is probably one of the main factors leading to unstable plaque, and NF-κB is the key transcription factor which causes inflammatory reactions. So NF-κB may play an important role in the formation of atherosclerosis and plaque disruption. By observing NF-κB expression of patients with ACS (a kind of acute CHD) before and after treatment, this paper’s author discusses the meaning of NF-κB in diagnosis and treatment of patients with ACS.

1 Material and Methods
1.1 Study Subject
Taking patients who received coronary angiography in cadre's wards of our hospital during from September 2010 to November 2011 as the sources of cases, and according to the order of admission to our hospital, choosing 88 patients with ACS consisting of 68 patients with unstable angina pectoris and 20 patients with acute myocardial infarction as the ACS group, 25 males, 18 females, age (67.3±5.6); the control group included 56 patients (45 men and 11 women, age from 61.6 to 71.4 ) with normal coronary angiogram who matched with the above-mentioned patients in age, sex, body mass index and smoking status, etc. All selected patients with CHD conformed to the WHO diagnostic standard. Excluding the following patients: (1) patients with acute infection or traumas and patients performed an operation within 4 weeks; (2) patients with amalgamative cerebral blood-vessel accident and peripheral arterial disease; (3) patients hepatic and renal dysfunction and malignant tumors. When they were selected, we informed all participants the experimental procedure and purpose, and obtaining their consent.

1. 2 Main instruments and reagents
Bayer 1650 full-automatic biochemical analyzer; CRP quick analyzer (Orion Diagnostica Company in Finland); FACS Calibur flow cytometer (American Becion Dickinson Company); Cycle TEST Plus DNA Kit (American Becion Dickinson Company); NF-κBP65 McAb(Santa Cruz Company in U.S.); IgG marked by FITC(American Jackson Company).
1.3 Methods
1.3.1 Blood lipid, blood sugar, liver function, high-sensitivity C-reactive protein (hs-CRP) testing

The fasting blood taken from cubital veins of the patients in two groups in the next morning after admission was sent to the clinical laboratory, in order to determine the indicators such as blood lipid, blood sugar, liver function, and hs-CRP, etc; after patients in ACS group treated with crown expansion drugs, lipid-lowering drugs and platelet aggregation inhibitors for a month, reviewing their index of blood lipid, blood sugar, liver function, and hs-CRP.

1.3.2 Detection of NF-κB

Taking cubital venous blood of these patients after admission for two hours, and pouring the sodium citrate injection for anticoagulant into the latter 3 ml to 4 ml which was used for testing NF-κB after discarding the first 2 ml of blood. Taking 50ul of the specimen, adding 2 ml of the hemolysin, placing for 30 minutes at room temperature after shaking well, throwing the supernatant away after centrifuging them twice in five minutes by the speed of 2000rpm/min, and then adding 1ml PBS liquor, pouring 200ul A liquor after shaking well, adding 100ul B liquor after 10 minutes’ standing, centrifuging them again after placing for 10 minutes in the same way, and eventually obtaining the single cell suspension which was necessary. Adding 0.2ml PBS liquor into ready-made suspension, changing the cell number as 1.0×10⁶/ml, dyeing the 10ul suspension combined with Wright Giemsa once, and observing in order to ensure the suspension successfully prepared under the microscope. Adding NF-κB p65McAb (diluted with saline water at a ration of 1 to 100) into cell suspension, placing for an hour at room temperature, adding IgG40ul (diluted with saline water at a ration of 1 to 100) marked by FITC and placing for 30 minutes, placing them again in the darkroom in 30 minutes after adding 20ul PI liquor, testing them through the computer, and detecting the NF-κB expression. Reviewing NF-κB of patients in ACS group after treatment with crown expansion drugs, lipid-lowering drugs, platelet aggregation inhibitors and anticoagulants for a month.

1.4 Statistical Methods

Taking SPSS 13.0 software package in statistical treatment, indicating measurement data by means of (x±s), and expressing the count index as a percentage. The difference was statistically significant (P<0.05).

2 Results

2.1 Comparisons of general information

There was no significant difference in age, sex, high blood pressure, hyperlipemia, and smoking of patients in two groups, P>0.05. But the ratio of diabetics in ASC group was higher than that of control group, the difference was statistically significant, P<0.01. (See Table 1)

Table 1 comparisons of general information in two groups of patients ( x±s)

<table>
<thead>
<tr>
<th>groups</th>
<th>n</th>
<th>female/male</th>
<th>Age (years old)</th>
<th>body weight index (BMI)</th>
<th>Hypertension (%)</th>
<th>diabetes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control group</td>
<td>56</td>
<td>47/11</td>
<td>63.5±7.6</td>
<td>24.6±3.2</td>
<td>18(31.0)</td>
<td>3(5.1)</td>
</tr>
<tr>
<td>ACS group</td>
<td>88</td>
<td>63/15</td>
<td>62.3±8.6</td>
<td>25.8±2.7</td>
<td>22(28.2)</td>
<td>18(23.1)</td>
</tr>
</tbody>
</table>

Note: in contrast with control group, * P < 0.01

2.2 Comparisons of TC, TG, LDL-C, levels of NF-κB activity and hs-CRP between two groups

The difference of blood lipid was not statistically significant, P > 0.05; the ACS group was markedly higher than control group in comparison of levels of NF-κB activity and hs-CRP, and the difference was statistically significant, P < 0.01. (See Table 2)

Table 2 comparisons of TC,TG,LDL-C,levels of NF-κB activity and hs-CRP between two groups (x±s)

<table>
<thead>
<tr>
<th>groups</th>
<th>TC (㎎/L)</th>
<th>TG (㎎/L)</th>
<th>LDL-C (mmol/L)</th>
<th>Positive rate of NF-κB (%)</th>
<th>hs-CRP (mg/L))</th>
</tr>
</thead>
<tbody>
<tr>
<td>control group</td>
<td>6.18±0.7</td>
<td>1.64±0.6</td>
<td>3.06±0.4</td>
<td>0.5±0.2</td>
<td>2.86±0.2</td>
</tr>
<tr>
<td>ACS group</td>
<td>6.56±0.6*</td>
<td>1.63±0.8*</td>
<td>3.63±0.6*</td>
<td>34.5±0.5**</td>
<td>18.87±0.5***</td>
</tr>
</tbody>
</table>

Note: in contrast with control group, * P > 0.05; **P < 0.01; *** P < 0.01

2.3 Comparisons of ASC group before and after drugs treatment

Blood lipids, levels of NF-κB activity and hs-CRP dropped significantly, and there was a statistically significant difference, P< 0.01. (See Table 3)
atherosclerosis inhibiting anti proved that NO has the effects of vasodilation, eventually form NO. In recent years, basic studies have of nitrosothiols via deamination, which generate a series conditions and prognoses of patients in ACS group.

Detecting the level of N of atherosclerosis and plaque disruption. NF activity and angiogram, with ACS and 58 patients and probably due to the decline of anti NF factor in the inflammatory process which is partially local inflammation with the result of producing pathway of mitogen inflammatory reactions. The content of lipid and inflammatory chemokine and tissue damage has occurrence of acute ischemic events. The effect of the connective tissue inflammatory reactions. The content of lipid and plaque and whether it is easy to result in plaque rupture of coronary atherosclerosis accompanied with mural thrombus caused by activating inflammatory reactions. The content of lipid and connective tissue in plaques determines the stability of the plaque and whether it is easy to result in the occurrence of acute ischemic events. The effect of inflammatory chemokine and tissue damage has admittedly been main determining factors of unstable lesions. These cytokines were generated from the signal peptide through TLRs which activate NF-kB and the pathway of mitogen-activated protein kinase (MAPK) with the result of producing cytokines to strengthen local inflammation.

The endothelial dysfunction associated with the increase of oxidative stress is an important initiating factor in the inflammatory process which is partially adjusted by NO, and this role is mainly mediated by NF-kB. So the vessel endothelial dysfunction is probably due to the decline of anti-inflammatory and anti-oxidative capabilities which result in the instability and erosion of plaques.

By contrastively researching blood lipids, levels of NF-kB activity, and hs-CRP of 78 patients with ACS and 58 patients with normal coronary angiogram, this study shows that levels of NF-kB activity and hs-CRP of patients in ACS group are significantly higher than those in control group, and NF-kB likely plays an important role in the formation of atherosclerosis and plaque disruption. Hence, detecting the level of NF-kB activity has a certain clinical significance in the evaluation of disease conditions and prognoses of patients in ACS group.

Nitrate esters after entering the body shape nitrosothiols via deamination, which generate a series of reactions through the membrane surface system and eventually form NO. In recent years, basic studies have proved that NO has the effects of vasodilation, anti-thrombus, anti-inflammation, anti-oxidation, and inhibiting vascular smooth muscle and prevents against atherosclerosis by these functions which are all clearly linked with NF-kB signal pathway. Ortege and other people firstly discovered atorvastatin could suppress the activation of NF-kB in vitro experiment. Ghosh and others found by studies aspirins could inhibit NF-kB from being degraded, which reserves NF-kB in the cytoplasm and avoids the activation of NF-kB. In addition, aspirins can be able to reduce adhesion molecules and the expression of macrophage chemotactrant protein-1, inhibit granulocytes from adhering to blood vessel endothelium and plaque, block plaque inflammation reactions, and prevent plaque disruption. Now research finds heparin is probably effective in the therapy of inflammatory diseases, and through chromatography or enzymatic synthesis of unfractionated heparin, low-molecular-weight heparin is depolymerized into glycopolypeptide with negative charge which plays a role in anti-coagulation, anti-inflammation, lowering lipid, and neuroprotection.

This study shows that their levels of NF-kB activity and hs-CRP significantly lower as compared with those before treatment after patients in ACS group treated with crown expansion drugs, lipid-lowering drugs and platelet aggregation inhibitors for a month, which proves that crown expansion drugs, lipid-lowering drugs and platelet aggregation inhibitors can reduce the level of NF-kB activity and stabilize plaque, and the level of NF-kB activity has a certain value on assessing the curative effect in the treatment of CHD and ACS.

The Fund Program
The scientific and technological brainstorm project of Henan province (No. 092102310065).

Corresponding author
Wang Lixia, chief physician, research on prevention and cure for coronary artery disease, Henan Provincial People's Hospital, Zhengzhou, Henan 450003, China E-mail: mingqinduan@sina.com

Table 3 comparisons of Blood lipids, levels of NF-κB activity and hs-CRP in ASC group before and after drugs treatment ( ± s)

<table>
<thead>
<tr>
<th>groups</th>
<th>TC (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>LDL-C (mmol/L)</th>
<th>Positive rate of NF-κB (%)</th>
<th>hs-CRP (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS group (before therapy)</td>
<td>6.56±0.6</td>
<td>1.63±0.8</td>
<td>3.68±0.6</td>
<td>34.5±0.5</td>
<td>18.87±0.5</td>
</tr>
<tr>
<td>ACS group (after therapy)</td>
<td>3.56±0.5*</td>
<td>1.53±0.4</td>
<td>2.63±0.4*</td>
<td>10.5±0.7**</td>
<td>9.87±0.5***</td>
</tr>
</tbody>
</table>

Note: in contrast with those before therapy, * P < 0.01; **P < 0.01; *** P < 0.01

3 Discussions
Recent years' research has indicated that the major pathogenesis of ACS is probably related to plaque rupture of coronary atherosclerosis accompanied with mural thrombus caused by activating inflammatory reactions. The content of lipid and connective tissue in plaques determines the stability of the plaque and whether it is easy to result in the occurrence of acute ischemic events. The effect of inflammatory chemokine and tissue damage has admittedly been main determining factors of unstable lesions. These cytokines were generated from the signal peptide through TLRs which activate NF-kB and the pathway of mitogen-activated protein kinase (MAPK) with the result of producing cytokines to strengthen local inflammation.

The endothelial dysfunction associated with the increase of oxidative stress is an important initiating factor in the inflammatory process which is partially adjusted by NO, and this role is mainly mediated by NF-kB. So the vessel endothelial dysfunction is probably due to the decline of anti-inflammatory and anti-oxidative capabilities which result in the instability and erosion of plaques.

By contrastively researching blood lipids, levels of NF-kB activity, and hs-CRP of 78 patients with ACS and 58 patients with normal coronary angiogram, this study shows that levels of NF-kB activity and hs-CRP of patients in ACS group are significantly higher than those in control group, and NF-kB likely plays an important role in the formation of atherosclerosis and plaque disruption. Hence, detecting the level of NF-kB activity has a certain clinical significance in the evaluation of disease conditions and prognoses of patients in ACS group.

Nitrate esters after entering the body shape nitrosothiols via deamination, which generate a series of reactions through the membrane surface system and eventually form NO. In recent years, basic studies have proved that NO has the effects of vasodilation, anti-thrombus, anti-inflammation, anti-oxidation, and inhibiting vascular smooth muscle and prevents against atherosclerosis by these functions which are all clearly linked with NF-kB signal pathway. Ortege and other people firstly discovered atorvastatin could suppress the activation of NF-kB in vitro experiment. Ghosh and others found by studies aspirins could inhibit NF-kB from being degraded, which reserves NF-kB in the cytoplasm and avoids the activation of NF-kB. In addition, aspirins can be able to reduce adhesion molecules and the expression of macrophage chemotactrant protein-1, inhibit granulocytes from adhering to blood vessel endothelium and plaque, block plaque inflammation reactions, and prevent plaque disruption. Now research finds heparin is probably effective in the therapy of inflammatory diseases, and through chromatography or enzymatic synthesis of unfractionated heparin, low-molecular-weight heparin is depolymerized into glycopolypeptide with negative charge which plays a role in anti-coagulation, anti-inflammation, lowering lipid, and neuroprotection.

This study shows that their levels of NF-kB activity and hs-CRP significantly lower as compared with those before treatment after patients in ACS group treated with crown expansion drugs, lipid-lowering drugs and platelet aggregation inhibitors for a month, which proves that crown expansion drugs, lipid-lowering drugs and platelet aggregation inhibitors can reduce the level of NF-kB activity and stabilize plaque, and the level of NF-kB activity has a certain value on assessing the curative effect in the treatment of CHD and ACS.

The Fund Program
The scientific and technological brainstorm project of Henan province (No. 092102310065).

Corresponding author
Wang Lixia, chief physician, research on prevention and cure for coronary artery disease, Henan Provincial People's Hospital, Zhengzhou, Henan 450003, China E-mail: mingqinduan@sina.com

686
References


