Abstract. – OBJECTIVE: To investigate the mechanism of exogenous adiponectin in the prevention of no-reflow phenomenon in type 2 diabetic (T2DM) patients with acute myocardial infarction (AMI) during percutaneous coronary intervention (PCI) treatment.

PATIENTS AND METHODS: 66 patients were randomly divided into control group and observation group, 33 cases in each group. According to the percutaneous coronary intervention (PCI) emergency treatment principle, patients from the control group were treated with an intracoronary injection of adenosine combined with a micro-pump intravenous infusion of tirofiban. Patients from the observation group were injected with exogenous adiponectin in addition to the adenosine and tirofiban treatments.

RESULTS: There were no significant differences in gender, age, location of the target lesion, degree of stenosis, stent implantation number, length and the inner diameter between control and observation group (p > 0.05). Lower frequent of slow blood flow and no-reflow and shorter interventional procedures were observed in observation group compared with those of control group (p < 0.05). Moreover, the increase of plasma creatine kinase (CK-MB) in patients of observation group was lower than that of the patients in control group (p < 0.05). In addition, the levels of troponin-I (cTnI), IL-6, TNF-α, endothelin-1 (ET-1), vascular endothelial adhesion molecular I (VCAM-1) and bax/Bcl-2 were significantly lower in observation group than those in control (p < 0.05). Furthermore, the occurrence of major adverse cardiac events (MACE) during a 12-month follow-up was significantly lower in the observation group than that of control (p < 0.05).

CONCLUSIONS: Exogenous adiponectin further reduced the no-reflow phenomenon during PCI treatment of the patients with T2DM combined with AMI. The function of exogenous adiponectin is associated with the reduced myocardial and endothelial cell injury and the inhibited inflammation and apoptosis. The application of exogenous adiponectin can significantly improve the clinical outcomes.

Key Words: Exogenous adiponectin, Type 2 diabetes mellitus, Acute myocardial infarction, No-reflow, Inflammation, Apoptosis, Major adverse cardiac events.

Introduction

Acute myocardial infarction (AMI) is a type of coronary heart diseases that causes high morbidity and mortality rates AMI can tremendously affect heart condition. Percutaneous coronary intervention (PCI) and stent implantation, which are two important strategies for the early treatment of AMI, have been confirmed to be effective in both short-term and long-term treatment1. No-reflow and slow blood flow, which occur in 6.5-25.8% of patients during an emergency and selective PCI, are risk factors for myocardial perfusion and ventricular remodeling2. Patients with no-reflow tend to have higher occurrences of long-term heart failure. This is likely associated with micro-thrombosis, myocardial apoptosis, myocardial stunning, inflammatory response, oxidative stress, calcium overload and microcirculation disorders3,4.

Coronary artery injection of tirofiban, nitroglycerin and adenosine can be used to reduce or even prevent the occurrence of no-reflow during emergency PCI treatment. Statin therapy is rel-
atively more effective than other therapies for the selective PCI treatment\textsuperscript{5,6}. It is known that the level of adiponectin was associated with the occurrence of AMI both in animal model experiments and clinical trials\textsuperscript{7,8}. Adiponectin, which is a cytokine secreted by adipocytes, has been proved to be endocrine effect. Adiponectin is also likely to be an important myocardial protective factor that has the functions of insulin-sensitizing, glycolipid metabolism regulation, anti-inflammatory and anti-ischemic injury and so on\textsuperscript{9}. It’s believed that the application of exogenous adiponectin might be able to reduce myocardial ischemia-reperfusion injury and prevent no-reflow phenomenon\textsuperscript{10}. However, little research has been done in humans.

With a globular head domain (gAd) as the active ingredient, recombinant human adiponectin can be generated by genetic engineering. After purification, recombinant human adiponectin has been proved to be safe for the application in humans\textsuperscript{11}. Our study aimed at investigating the function of exogenous adiponectin in the prevention of no-reflow in patients with type 2 diabetes mellitus (T2DM) combined with AMI during PCI, so as to provide the basis for the theoretical studies and clinical treatment of no-flow.

**Patients and Methods**

**Patients**

66 patients who were admitted to Central Hospital of EnShi from March 2014 to September 2015 were selected. These patients were diagnosed with T2DM combined with AMI. The selection was made according to the following inclusion criteria: patients aged between 18 to 70 years old; patients with emergency PCI indications, complete clinical data and informed consent obtained. Exclusion criteria: patients that have been treated with thrombolytic therapy; patients with a risk of bleeding, severe complications of diabetes, and contrast medium sensitivity; patients with liver and kidney dysfunction, adiponectin intolerance, that failed in PCI treatment, or with serious complications. The study was approved by the Ethics Committee of Central Hospital of EnShi Autonomous Prefecture.

In accordance with the order of admission, the patients were randomly divided into control group and observation group (33 cases for each group). There were 20 males and 13 females in the control group, with an average age of (58.2 ± 15.5) years, a mean duration of diabetes mellitus of (3.5 ± 1.2) years, and a mean duration of AMI of (7.3 ± 2.5) hours. ST-elevation myocardial infarction (STEMI) was found in 25 cases; non-STEMI (NSTEMI) was found in 8 cases. There were 18 males and 15 females in the observation group, with an average age of (56.7 ± 13.8) years, a duration of diabetes of (3.3 ± 1.1) years, and a mean duration of AMI onset of (7.1 ± 2.3) hours. There were 23 cases of STEMI and 10 cases of NSTEMI. No significant difference in gender, age, duration of diabetes, duration of diabetes AMI and type of AMI was found between the two groups.

**Methods**

**Standard PCI Technique**

Standard PCI techniques were applied as follows: complete preoperative examination, oral intake clopidogrel 300 mg and aspirin 300 mg, and whole heparinization. A conventional Seldinger technique was used to puncture the right radial artery, followed by selective coronary angiography and infarct-related artery (IRA). The appropriate interventional transport equipment was selected to place a stent; the angiography was reviewed. The criteria for the successful PCI treatment: TIMI grade 3 under direct vision. In the control group, intracoronary injection of adenosine and intravenous infusion of tirofiban were performed. 24-48 μg adenosine were administered by bolus injection before or after each balloon dilatation. Tirofiban hydrochloride (Xinweining, China Grand Pharmaceutical Co., Ltd.) was infused intravenously for 10 min with a loading dose of 5 mg, followed by the speed of 5 μg/kg/min by a micro pump. For the observation group, the intracoronary bolus injection of 10 μg exogenous adiponectin was completed before the dilation of the balloon and after the injection of adenosine.

**Preparation of Globular Domain Adiponectin (gAd)**

gAd is produced by the Shanghai Megui Biological Technology Co., Ltd. Production, No.: RD001-01. Total RNA was extracted from human visceral adipose tissue and reverse transcribed into cDNA. Adiponectin cDNA fragment was amplified by PCR. After PCR amplification using gAd specific primers, the PCR product was ligated with pET22b (+) vector to construct recombinant
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plasmid by Nde I and EcoR I double digestion. The recombinant plasmid was then transferred into *Escherichia coli* BL21 (DE3) competent cells to induce the expression of the target protein. The recombinant protein was produced in the form of inclusion body and dissolved in strong alkaline solution. After re-naturalization and purification by acetone precipitation method, recombinant human gAd with high purity was generated. *In vivo* and *in-vitro* experiments were performed to confirm that the recombinant human gAd has low immunogenicity but high biological activity.

**Statistical Analysis**

Statistical analysis was completed with SPSS 20.0 software (IBM, Armonk, NY, USA) and all quantitative data were expressed as the mean ± standard deviation. An independent sample *t*-test was used for the comparison between groups. Paired *t*-test was used for the comparisons within one group. The counting data were expressed as cases/percentage. The comparison between groups was analyzed by the *χ*²-test. *p* < 0.05 indicated that the difference was statistically significant.

**Results**

No significant differences in sites and numbers of target lesion, stenosis severity, number of stents implanted, length and diameter of the stent, prevalence rate of slow blood flow and no-reflow, and time of intervention (the first balloon dilatation to the end of treatment) were found between the control and observation groups. The increase of creatine kinase isoenzyme (CK-MB) and troponin-I (cTnI) during surgery was defined as [(post-surgery-presurgery) / presurgery × 100%]; the levels of IL-6, TNF-α, ET-1, VCAM-1 and bax/Bcl-2 were also compared between the two groups.

The occurrence rates of major adverse cardiac events (MACE) were observed throughout 12.0-month follow-up. The levels of IL-6, TNF-α, ET-1, VCAM-1, Bcl-2 and bax in plasma were detected by ELISA. Reagents for IL-6 and TNF-α were obtained from Jiangsu Biyun Tian Technology Co., Ltd (Jiangsu, China). ET-1 and VCAM 1 were acquired from Beijing Zhongsheng Jinqiao Biology Co., Ltd (Beijing, China); Bcl-2 and bax were purchased from Sigma-Aldrich (St. Louis, MO, USA); CK-MB and cTnI were purchased from Invitrogen Corporation (Carlsbad, CA, USA). The automatic biochemical analysis was conducted on Olympus AU400 (Tokyo, Japan).

A comparison of the sites and numbers of the target lesion, stenosis severity, number of stents implanted, as well as the length and the diameter of the stent between the two groups was made. Differences in sites and numbers of the target lesion, stenosis severity, number of stents implanted, as well as the length and the diameter of the stent between the two groups, were all not significant (Table I).

A comparison of the occurrence rates and the intervention time of slow-flow/no reflow between two groups was made: the prevalence rates of both slow-flow and no-reflow were reduced and the intervention time was shortened significantly in the observation group compared with those in control group (*p* < 0.05) (Table II). The difference in the increase of CK-MB and cTnI, as well as the levels of IL-6 and TNF-α between the two groups during surgery, were also compared. The increase of CK-MB and cTnI, as well as the levels of IL-6 and TNF-α during surgery, were significantly lower in the observation group compared with those in the control group (*p* < 0.05) (Table III). The comparison of ET-1/VCAM-1 and bax/Bcl-2 levels in plasma was also performed: the levels of ET-1/VCAM-1 and bax/Bcl-2 were significantly lower in the observation group compared with those in the control group (*p* < 0.05) (Table IV). In the comparison of the occurrence rates of MACE during 12-month follow-up, the occurrence rate of MACE was lower in the observation

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**Table I.** Two groups are compared with respect to sites, number of lesion, number of target lesions, number of stents involved, stenosis activity, and length of the diameter of stent.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>AD</th>
<th>CX</th>
<th>RCA</th>
<th>Number</th>
<th>Stenosis severity (%)</th>
<th>Number of stents</th>
<th>Length (mm)</th>
<th>Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>33</td>
<td>15</td>
<td>6</td>
<td>14</td>
<td>1.1 ± 0.4</td>
<td>92.5 ± 3.7</td>
<td>1.3 ± 0.5</td>
<td>35.6 ± 5.4</td>
<td>24.6 ± 4.7</td>
</tr>
<tr>
<td>Observation</td>
<td>33</td>
<td>17</td>
<td>5</td>
<td>15</td>
<td>1.2 ± 0.5</td>
<td>94.3 ± 3.9</td>
<td>1.5 ± 0.7</td>
<td>33.5 ± 5.8</td>
<td>22.8 ± 4.9</td>
</tr>
<tr>
<td><em>t</em>/<em>χ</em>²</td>
<td>0.195</td>
<td>0.092</td>
<td>0.254</td>
<td>0.196</td>
<td>0.203</td>
<td>0.245</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>p</em></td>
<td>0.907</td>
<td>0.953</td>
<td>0.867</td>
<td>0.869</td>
<td>0.867</td>
<td>0.823</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

AD: anterior descending branch; CX: circumflex branch; RCA: right coronary artery.
group during 12-month follow-up compared with that in the control group (p < 0.05) (Table V).

Discussion

Myocardial ischemia-reperfusion injury manifests itself through slow blood flow and no reflow. In this study, it was assumed that exogenous adiponectin could decrease the occurrence of myocardial ischemia-reperfusion injury. Previous studies\(^1\) have confirmed that reduced adiponectin secretion can increase the occurrence of obesity-induced insulin resistance and type 2 diabetes as well as the severity of AMI myocardial injury and ischemia-reperfusion injury. We found that the prevalence rates of both slow flow and no-reflow were reduced and the intervention time was shortened in the observation group compared with those in the control group. Also, the levels of the increase of plasma CK-MB and cTnI, IL-6, TNF-α, ET-1, VCAM-1, and bax/Bcl-2 in the observation group were significantly lower than those in the control group (p < 0.05). The interventional operation itself can increase the inflammation of the coronary lesion site, induce micro-thrombosis, cause endothelial dysfunction, and increase cell apoptosis and necrosis\(^3\).
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Also, stent network structure can increase microcirculation dysfunction, induce chemotaxis, and activate monocytes to release a variety of vasoactive factors such as ET-1, and adhesion molecules such as VCAM-1, which, in turn, participate in the occurrence of reperfusion injury. Although PCI can significantly reduce thrombus load, it can also inhibit smooth muscle cell proliferation and disrupt the endothelial cell microcirculation, leading to the occurrence of no-reflow. IL-6 and TNF-α are important cytokines in the inflammation response that can promote the release and activation of other inflammatory cells and mediators. IL-6 and TNF-α also play important roles in the damage of coronary endothelial cells and the migration of macrophages.

The occurrence of coronary heart disease is closely related to lipid deposition and inflammatory response. The inflammatory response can enhance lipid deposition and plaque rupture, which in turn cause thrombosis and bleeding. The balance between apoptotic bax and antiapoptotic Bcl-2 is involved in myocardial injury and ventricular remodeling. The significant reduction in the occurrence of MACE in observation group observed during follow-up indicated that the application of exogenous adiponectin could reduce the prevalence of no-reflow and improve the long-term clinical outcome.

Researches have suggested that adiponectin can be used as a sensitive early marker to predict the occurrence of coronary heart disease in patients with T2DM. The full-length adiponectin contains the amino-terminal signal sequence, the variable region, the collagen-like region and the carboxy-terminal globular domain (gAd). The gAd exists in normal human blood, and exogenous injection of gAd can decrease glucose and free fatty acid levels in plasma and improve insulin resistance. Although the concentration of gAd is low, the activity of gAd is stronger than that of the full-length adiponectin. gAd can be engineered and expressed in E. coli, yeast and mammalian cells (HEK293-T cells). Further researches aiming at large-scale production, improving protein purity and in vivo activity, as well as reducing heterogeneity, are needed.

Conclusions

Exogenous adiponectin has significant value in reducing the no-reflow phenomenon occurred during emergency PCI treatment of T2DM patients with AMI. The effects of adiponectin are related to its function in alleviating myocardial and endothelial cell injury, and inhibiting inflammation and apoptosis. The application of exogenous adiponectin will benefit long-term clinical outcomes.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

9) Zhao L, Chai W, Fu Z, Dong Z, Taylor KW, Barrett EJ, Cao W, Liu Z. Globular adiponectin enhances
C.-J. Zhang, Y.-Z. Deng, Y.-H. Lei, J.-B. Zhao, W. Wei, Y.-H. Li


