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The mechanism of exogenous adiponectin in the prevention of no-reflow phenomenon in type 2 diabetic patients with acute myocardial infarction during PCI treatment

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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, 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 significances in gender, age, location of the target lesion, degree of thrombosis, stent implantation number, length, and inner diameter between control and observation group ($p > 0.05$). Lower frequency of slow blood flow and no-reflow and shorter intervention procedures were observed in observation group compared with those in control group ($p < 0.05$). Moreover, the increase of plasma creatine kinase (CK-MB) in patients of observation group was lower than that of patients in control group ($p < 0.05$). Additionally, levels of troponin-I (cTnI), IL-6, TNF-α, endothelin-1 (ET-1), vascular endothelial cell 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 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 treatment[1]. 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 remodeling[2]. 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 disorders[3,4].

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-

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atively more effective than other therapies for the selective PCI treatment[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[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[9]. It's believed that the application of exogenous adiponectin might be able to reduce myocardial ischemia-reperfusion injury and prevent no-reflow phenomenon[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[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 d... PCI, so as to provide the basis for the the... studies and clinical treatment of no-flow.

Patients and Me...

Patients

66 patients who w... Hospital of EnShi f... to Septem-ber 2015 were sel...d. These p...ts were diagnosed with T...ombined w...MI. The selection was ...ade ...ding to th... following inclusion ...ria: patie...ged between 18 to 70 yea...d; patients wit...ergency PCI indicat..., comp...te clinical ...ata and informed co... obtai... Exclusion criteria: patients that have ...ated w... thrombolytic therapy; ...ients ...a risk ...eeding, severe complica-t...of di...d contrast medium sensitivity ...tients ...liver and kidney dysfunction, a...in intolerance, that failed in PCI treatment, ...n serious complications. The study ...as approved by the Ethics Committee of Central ...ital of EnShi Autonomous Prefecture.

...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 ob... group, with an average age of (56.7 ± ...) years, a duration of diabetes of (3.3 ± 1.... ...ars, and a mean duration of AMI onset of (7.1 ...) hours. There were 23 cases of STE... and ...es of NSTEMI. No significant ...erence in ... age, duration of diabe... durati... of dia... AMI and type of A... as fo... between the two groups.

Methods

Stan... PCI Tech...e

...dard PCI techniq... were applied as fol-l... complete preoperative examination, oral in-t... clopidogrel ... mg and aspirin 300 mg, and w... heparin... n. A conventional Seldinger tec... was ...d to puncture the right radial art...ed by selective coronary angiography and infarct-related artery (IRA). The ...riate interventional transport equipment ...cted 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

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 numb of target lesion, stenosis severit of sten implanted, length and diame the nt, prev- alence rate of slow blood and no low, and time of intervention (the ball to the end of treatm were between the control and obser n groups. increase of creatine kinase me (CK-M d tropo- nin-I (cTnI) d g su was defin as [(post- surgery-pr gery) / p ery × 100%]; the levels -6, TNF-α, ET CAM-1 and bax/ Bcl-2 e also pared betw en the two groups. occu e rates of major adverse car- diac MACE re observed through- 12.0 nth f -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. China); Bcl-2 and bax were purchas om Sig ma-Aldrich (St. Louis, MO, USA -MB and cTnI were purchased from Invitrog rpora- tion (Carlsbad, CA, USA). auto bio- chemical analysis was c cted on C AU400 (Tokyo, Japan).

A comparison of t s an mbers of the target lesion, sten s mber tents implanted, as w the le and th ameter of the stent h n the two as made. Difference s and numbe the target lesion, steno sis se number of stents im- plante well as th gth and the diameter of tent between the groups, were all not s ficant (Table I).

comparison the occurrence rates and the i ention tim slow-flow/no reflow between two ps w ade: the prevalence rates of both s and no-reflow were reduced and the intervention time was shortened significantly observation group compared with those in 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-1VCAM-1 and bax/Bcl-2 levels in plasma was also performed: the levels of ET-1VCAM-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

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

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

AD: anterior descending branch; CX: circumflex branch; RCA: right coronary artery.

Table II. Comparison of the occurrence rates and the intervention time of slow-flow/no reflow between two groups.

Groups	Cases	Slow-flow	No-reflow	Occurrence rates of slow-flow and no-reflow (%)	Intervention time (min)
Control	33	6	4	10 (30.3)	5.6 ± 1.8
Observation	33	2	1	3 (9.1)	2.2 ±
t/χ^2	–	–	–	4.694	
p	–	–	–	0.030	06

Table III. Comparison of the rise in CK-MB and cTnl levels as well as the levels of IL-6 and TNF-α between two groups during surgery.

Groups	Increase of CK-MB	Increase of cTnl	IL-6 (mmol/L) before surgery	IL-6 in surgery	TN (mmol/	TNF-α in surgery
Control	1.1 ± 0.3	0.9 ±0.2	156.4 ± 45.7	114.7 ± 31.2	56.5	± 8.7
Observation	0.8 ± 0.2	0.7 ± 0.2	162.3± 49.8	85.2 ± 20	57.2 ± 1	8.5 ± 6.2
t	3.659	3.458	0.262	6.52	0.193	6.128
p	0.031	0.035	0.865	0.	0.902	0.000

Table IV. Levels of plasma ET-1, VCAM-1 and bax/Bcl-2 plasma.

Groups	ET-1 (μmol/L)		VCA (μmol/L)		bax/Bcl-2	
	Before surgery	In surgery	Before surge	In surg	Before surgery	In surgery
Control	165.8 ± 82.3	107.8 ± 56.2	± 75.8	5.4	0.66 ± 0.25	0.53 ± 0.23
Observation	173.3 ± 93.5	88.5 ± 35.4		112.3 ± 58.9	0.69 ± 0.29	0.38 ± 0.17
t	0.313	5.852	0.2	6.052	0.069	4.658
p	0.758	0.012	0.8	0.007	0.952	0.025

Table V. Comparison of the occ ce rates MACE dur ollow-up (%).

Groups	Cases	ent	ilure orse	Target blood vessel reconstruction	Death	Occurrence rate of MACE
Control		2	3	2	1	8 (24.2)
Observation		1	1	0	0	2 (6.1)
χ^2	–	–	–	–	4.243	
p	–	–	–	–	0.039	

gr uring month follow-up compared with that i rol gr $p < 0.05$) (Table V).

Discussion

Myoc l ischemia-reperfusion injury mani-sts itself through slow blood flow and no reflow. his study, it was assumed that exogenous a onectin could decrease the occurrence of myocardial ischemia-reperfusion injury. Previous studies[12] 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[13].

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[14]. 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[15]. 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[16].

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[17,18]. The balance between apoptotic bax and antiapoptotic Bcl-2 is involved in myocardial injury and ventricular remodeling[19]. The significant reduction in the occurrence of MACE in observ[illegible] group observed during follow-up indicat[illegible] the application of exogenous adiponectin [illegible]d reduce the prevalence of no-reflow and imp[illegible] the long-term clinical outcome.

Researches[20] have suggeste[illegible]iponect[illegible] can be used as a sensitive ea[illegible]mark[illegible] predict[illegible] the occurrence of corona[illegible]eart dis[illegible]e in patients with T2DM. Th[illegible]ngt[illegible] contains the amino[illegible]mina[illegible] sequence, the variable regio[illegible]e collage[illegible] region and the carboxy-te[illegible]globular d[illegible]in (gAd). The gAd exis[illegible] no[illegible] human blo[illegible]d, and exogenous i[illegible]tion of gA[illegible] decrease glucose and fre[illegible]y acid levels i[illegible]ma and improve insul[illegible]esistan[illegible]. Although the concentration of [illegible] is lo[illegible]e activity of gAd is stronger than [illegible]e full-le[illegible]h adiponectin. gAd can [illegible]engi[illegible]d and [illegible]pressed in *E. coli*, yeast a[illegible]amm[illegible]s (HEK293-T cells)[21]. Furthe[illegible] searche[illegible]ning at large-scale production, i[illegible] protein purity and *in vivo* activity, as [illegible]ll as [illegible]eing 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 ha[illegible] conflict of [illegible]ts

Refere[illegible]

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