Effects of remote ischemic post-conditioning on platelet activation of AMI patients

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Abstract. The recovery of ischemic myocardium blood perfusion is the main treatment option for acute myocardial infarction (AMI). However, this treatment option has multiple side effects that directly affect the quality of life of the patients. The activation of platelet function plays an important role in the occurrence, development and treatment of AMI. The aim of the present study was to analyze the effects of remote ischemic post-conditioning on platelet activation of AMI patients with primary PCI treatment and clinical prognosis. A total of 71 patients with AMI were treated with primary percutaneous coronary intervention (PCI). They were randomly divided into control group (n=34) and observation group (n=37). The patients in the observation group were treated with remote ischemic post-conditioning. Further, flow cytometer was used to detect the platelet alpha granule membrane glycoprotein (CD62P) and the percentages of activated IIb/IIIa (PAC-1). The maximum platelet aggregation rate induced by adenosine diphosphate (ADP) and arachidonic acid (AA) was measured by light transmittance aggrometer. The incidence of major adverse cardiac events (MACE) was compared between the two groups during the follow-up period of 6 months. The percentage of CD62P (24 h after PCI) in the observation group was significantly lower than control group (P<0.05). Further, the incidence of MACE in the observation group was also lower than that of the control group (P<0.05). Remote ischemic post-conditioning could reduce the incidence of MACE in patients with AMI after primary PCI treatment. Moreover, the above observation may be related to the improvement of platelet CD62P activation.

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Introduction

The timely opening of the culprit vessels and restoration of the blood perfusion of ischemic myocardium is the main treatment for acute myocardial infarction (AMI). It helps in maintaining heart function and improves clinical prognosis (1). However, reperfusion injury caused by percutaneous trans-luminal coronary angioplasty and percutaneous coronary intervention (PCI) results in poor blood flow as well as stent restenosis (2-4). The above factors collectively reduce the therapeutic effect and quality of life of AMI patients. Multiple studies have confirmed (5,6) that the activation of platelet function plays an important role in the occurrence, development and treatment of AMI. PCI pre-operative and post-operative antiplatelet therapy is the basis of AMI therapy.

Basic and clinical research supports *in situ* ischemic preconditioning (7,8) as it has ability to reduce the degree of platelet activation. On the other hand, the remote ischemic post-conditioning is different from *in situ* ischemic pre-conditioning. The clinical operation is simple and is quite easy (9,10). The aim of the present study was to analyze the effects of remote ischemic post-conditioning on platelet activation of AMI patients with primary PCI treatment and clinical prognosis.

Patients and methods

Patients. A total of 71 patients with the first diagnosis of acute ST-segment elevation myocardial infarction (STEMI) in The First Affiliated Hospital of Soochow University (Suzhou, China) from October, 2015 to May, 2016 were continuously selected. Inclusion criteria: i) PCI treatment performed within 90 min after first visit and ii) according to the research program, the clinical data were completed, and the hospital 's Ethics Approval and informed consents were obtained. Exclusion criteria: i) Patients with additional heart diseases such as dilated cardiomyopathy were excluded; ii) patients with combined pump failure with the need of mechanical ventilation treatment; iii) target lesions not suitable for PCI therapy, such as the left main branch, bifurcation lesions, and calcification lesions; iv) high risk of bleeding, and coagulation disorders were excluded and v) severe hepatorenal dysfunction.

According to the order of admission, the patients were randomly divided into control group (n=34) and observation

Table I. Baseline data of the two groups.

Variables	Control group (n=34)	Observation group (n=37)	t/χ²	P-value
Male/female	18/16	20/17	0.009	0.925
Age (years)	56.5±12.3	56.3±13.5	0.121	0.968
Smoking cases	10 (29.4%)	13 (35.1%)	0.265	0.607
Hypertension cases	19 (55.9%)	20 (54.1%)	0.024	0.877
Diabetes cases	3 (8.8%)	5 (13.5%)	0.390	0.532
Family history of coronary heart disease cases	2 (5.9%)	3 (8.1%)	-1.000	
Blood vessels			0.723	0.697
Anterior descending branch	19 (55.9)	18 (48.6)		
Right coronary artery	13 (38.2)	15(40.5)		
Circumflex	2(5.9)	4 (10.8)		
Platelet count (10^3/mm³)	207±85	205±48	0.135	0.902
Glutamic pyruvic transaminase (U/l)	74±36	63±31	0.425	0.369
Serum creatinine (μmmol/l)	87±22	77±18	0.326	0.518
Low density lipoprotein (mmol/l)	3.07±1.03	3.00±0.97	0.107	0.798
High sensitive C reactive protein (mg/l)	8.01±4.42	8.38±4.49	0.124	0.757
Use time of tirofiban (h)	20.9±9.5	22.4±9.8	0.213	0.664

group (n=37). The baseline data of the two groups are comparable (Table I).

Research methods. The coronary artery was opened as per standardized protocols by the surgical and nursing team. They were treated with pre-operative oral doses of aspirin 300 mg and clopidogrel 300 mg, post-operative oral doses of aspirin 100 mg/day and clopidogrel 75 mg/day. Further, intra-operative and post-operative short-term low dose treatment was of tirofiban (5 μg/kg/h).

Remote ischemic post-conditioning: The observation group used the cuff-of sphygmomanometer to tightly wrap the left arm of the patient, inflated to 200 mmHg and maintained for 5 min, and then deflated to 0 mmHg. The condition was maintained for 5 min. This was one cycle, and a total of four cycles were performed. The control group also used the sphygmo-manometer for inflation up to 10 mmHg for 5 min, and was then deflated to 0 mmHg. Similarly, 4 cycles were carried out.

Observation index. Before PCI, the moment of intervention, 24 and 48 h after PCI, respectively, flow cytometer was used to detect the platelet alpha granule membrane glycoprotein (CD62P) and the percentages of activated IIb/IIIa (PAC-1). The maximum platelet aggregation rate induced by adenosine diphosphate (ADP) and arachidonic acid (AA), were measured by light transmittance aggrometer. The incidence of major adverse cardiac events (MACE) was compared between the two groups during the follow-up period of 6 months. Venous blood samples 4-5 ml were collected from patients with 3.8% sodium citrate (1:9) anticoagulation. Platelet rich plasma took 200 x g centrifugation for 10 min. Platelet-poor plasma was centrifuged at 1,300 x g for 15 min. Platelet count of platelet rich plasma was adjusted to 3 times x 10^{11}/l.

Determination of CD62P and PAC-1. A total of 20 μl platelet-rich plasma was added to 5 μl FITC-labeled CD62P (BioLegend, San Diego, CA, USA) and incubated for 15 min at 37˚C in the dark. A total of 5 μl platelet rich plasma was added to 10 μl FITC-labeled PAC-1 (Becton-Dickinson, San Diego, CA, USA) and was then incubated for 15 min at 37˚C in the dark. Flow cytometer (Beckman XL; Beckman Coulter, Inc., Brea, CA, USA) was used for measurements.

Platelet maximum aggregation rate. Platelet rich plasma was incubated at 37˚C for 10 min. 10 μM ADP and 0.5 μM AA were used to induce platelet aggregation. The reagents were purchased from Jiangsu Biyuntian Biotechnology Co., Ltd. (Jiangsu, China).

Statistical analysis. SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Measurement data were expressed by mean ± standard deviation. Comparison between two groups was tested by independent sample t-test. Analysis of variance with Fisher's exact post hoc test of repeated measures was used for percentage of CD62P and PAC-1 comparison at different time-points. Countable data were expressed by the number of cases or (%). Comparisons among groups were by χ² method. P<0.05 indicated that the difference was statistically significant.

Results

Determination of CD62P and PAC-1. The percentage of CD62P (24 h after PCI) in the observation group was significantly lower than that in the control group (P<0.05). There was no difference at other time-points (P>0.05). Moreover, there was no statistical difference in the percentages of PAC-1 in both the groups at each time-point (P>0.05), (Table II; Fig. 1).
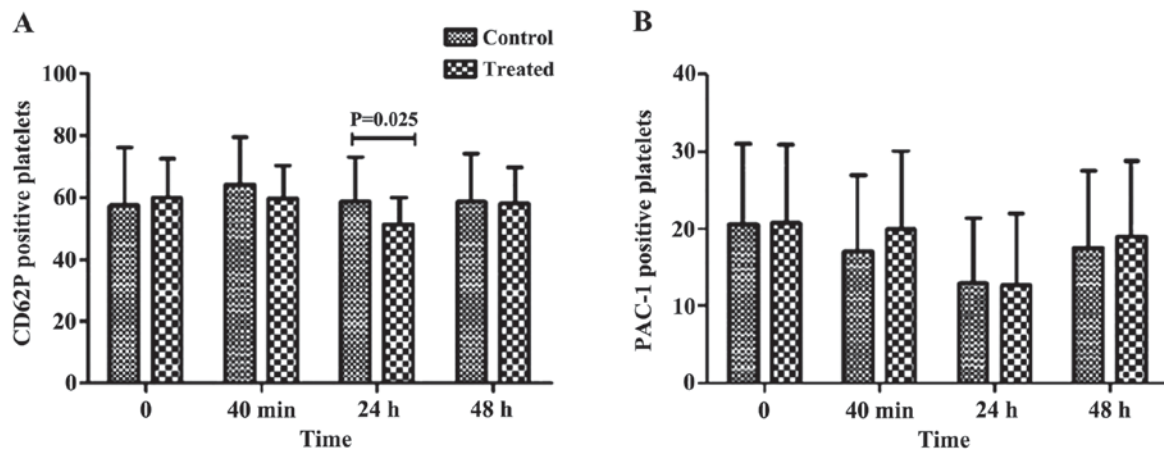
Figure 1. The percentage of CD62P and PAC-1 detected by flow cytometry. (A) The percentage of CD62 24 h after PCI in the observation group was significantly lower than that in the control group after PCI (P<0.05). (B) There was no difference in other time-points (P>0.05). There was no statistical difference in the percentage of PAC-1 in the groups at any time-point (P>0.05). CD62P, alpha granule membrane glycoprotein; PCI, percutaneous coronary intervention.

Table II. Determination of CD62P and PAC-1 (%).

Group	Control group (n=34)	Treatment group (n=37)	t-test	P-value
CD62P				
Before PCI	57.6±18.5	59.9±12.5	0.365	0.583
The moment of intervention	64.2±15.3	59.6±10.5	0.693	0.199
24 h after PCI	58.5±14.6	51.1±8.6	5.236	0.025
48 h after PCI	58.6±15.5	57.9±11.7	0.187	0.853
PAC-1				
Before PCI	20.6±10.3	20.8±10.1	0.065	0.972
The moment of intervention	17.1±9.8	19.9±10.1	0.587	0.571
24 h after PCI	12.9±8.4	12.6±9.4	0.132	0.911
4 h after PCI	17.5±10.0	18.9±9.7	0.365	0.721

CD62P, alpha granule membrane glycoprotein; PCI, percutaneous coronary intervention.

Table III. Determination of the maximum platelet aggregation rate (%).

Group	Control group (n=34)	Treatment group (n=37)	t-test	P-value
ADP				
Before PCI	44.8±6.4	44.6±6.3	0.047	0.953
The moment of intervention	20.0±6.9	23.8±6.3	0.623	0.458
24 h after PCI	13.8±5.9	21.0±6.5	0.421	0.679
48 h after PCI	24.0±6.5	26.1±5.6	0.359	0.621
AA				
Before PCI	28.7±7.0	30.1±6.2	0.212	0.881
The moment of intervention	14.7±4.9	25.1±6.5	0.724	0.210
24 h after PCI	5.8±2.3	3.0±1.9	0.863	0.132
48 h after PCI	7.1±1.9	5.2±1.3	0.659	0.222

ADP, adenosine diphosphate; PCI, percutaneous coronary intervention.

Determination of the maximum platelet aggregation rate. There was no significant difference in maximum platelet aggregation rate induced by ADP and AA at different time-points (P>0.05). The two groups reached the lowest 24 h after PCI, and recovered 48 h after PCI (Table III; Fig. 2).

Comparison of MACE. The incidence of MACE in the observation group was lower than that in the control group and the difference was statistically significant (P<0.05) (Table IV).

Discussion

The results of the present study showed that the percentage of CD62P after 24 h of PCI, in the observation group was significantly lower than that in the control group. Further, there was no difference in other time-points. Also, there was

no significant difference in maximum platelet aggregation rate induced by ADP and AA at different time-points. The incidence of MACE in the observation group was lower than that of the control group. Thus, it could be suggested that remote ischemic post-conditioning has the ability to reduce the incidence of MACE in patients with AMI after primary PCI treatment. Furthermore, this may be related to the improvement of platelet CD62P activation. Earlier studies (11,12) revealed that remote ischemic post-conditioning could increase the release of reactive oxygen species, adenosine, bradykinin, opioids and other factors. These factors in turn activated intracellular tyrosine kinase (TK), MEK1/2, PIK3, AKT, ERK1/2, so as to upregulate RISK reperfusion injury protective enzyme pathway (13,14). These factors could have mediated the downstream target molecules such as eNOS, GC in order to activate potassium ATP channel (15), and may have
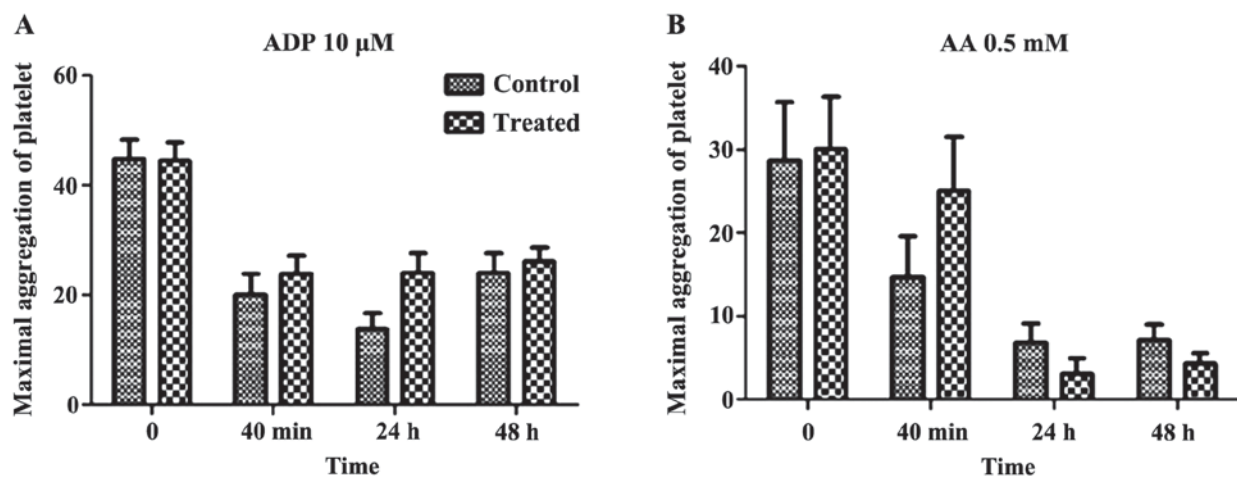
Figure 2. Maximum platelet aggregation rate detected by turbidimetry. There was no significant difference in maximum platelet aggregation rate induced by (A) ADP and (B) AA at different time-points (P>0.05). The two groups were at the lowest level 24 h after PCI, and recovered 48 h after PCI. PCI, percutaneous coronary intervention; ADP, adenosine diphosphate; AA, arachidonic acid.

Table IV. Comparison of MACE [cases (%)].

Group	Target vessel reconstruction	Recurrent myocardia infarction	Congestive heart failure	Rehospital-ization	Sudden cardiac death	Overall incidence
Control group (n=34)	1	2	2	3	1	9 (26.5)
Treatment group (n=37)	0	1	1	1	0	3 (8.1)
χ^2						4.254
P-value						0.039

MACE, major adverse cardiac events.

collectively caused inhibition of mitochondrial permeability transition pore (mPTP) opening (16). However, the specific mechanism needs further confirmation.

CD62P and PAC-1 are considered as the markers of platelet activation. PAC-1 is considered as a GPIIb/III complex fibrinogen receptor. After platelet activation, GPIIb/IIIa receptor activity expression is the final path to induce platelet aggregation. Thus, PAC-1 is also considered to be an early marker of platelet activation (17). On the other hand, CD62P is expressed in plasma alpha granules in resting state, when platelets and endothelial cells are stimulated by thrombin, histamine and oxygen free radicals. It has been considered as a specific marker of late platelet activation (18). The results of the present study showed that remote ischemic post-conditioning could decrease the expression of CD62P in platelets but had no effect on the expression of PAC-1. The reason could be primarily the treatment of the patients with triple antiplatelet therapy (aspirin + clopidogrel + tirofiban), and especially tirofiban was antagonist of the platelet GPIIb/IIIa receptor. This had a great influence on the expression of PAC-1. Secondly, the statistical difference of CD62P expression after 24 h may be the delayed protective effect of ischemic post-conditioning, and the early influence on platelet function may be masked by antiplatelet drugs. Thirdly, CD62P was a late specific marker of platelet activation, so there was a statistical difference between the two groups after 24 h.

Aspirin, clopidogrel and tirofiban affected platelet aggregation rates. Moreover, it was indicated that the remote ischemic post-conditioning had an effect on platelet activity, but its effect was far weaker than that of antiplatelet drugs. All of the participants in this study had the first occurrence of AMI, excluding the effect of ischemic pre-conditioning on the study results. Remote ischemic post-conditioning could further reduce platelet activity in patients with AMI, and could play a synergistic role with antiplatelet drugs. The prime drawback of the present study was the small sample size. Moreover, the mechanism of remote ischemic post-conditioning effect on platelet function was not analyzed in-depth. Subjects receiving multiple antiplatelet therapies may have an impact on the outcome of the study.

Competing interests

The authors declare that they have no competing interests.

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