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Relationship of platelet microparticle CD62P and activated GP IIb/IIIa with hypercoagulable state after atrial fibrillation radiofrequency catheter ablation

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Abstract. – OBJECTIVE: The morbidity of atrial fibrillation (AF) is 1%-2% in clinic. Radiofrequency catheter ablation (RFCA) is a type of radical interventional therapy for AF, whereas it may lead to a hypercoagulable state. This study evaluated platelet particle CD62P and platelet activation biomarker GP IIb/IIIa expressions in AF patients treated by RFCA, and aimed to analyze their relationships with the hypercoagulable state after RFCA.

PATIENTS AND METHODS: A total of 60 AF patients received RFCA in our hospital were enrolled. The patients were divided into group A as hypercoagulable state group and group B as non-hypercoagulable group. Healthy volunteers were selected as normal control. Serum D-Dimer, parathyroid activity index 1 (PAI-1), and tissue plasminogen activator (t-PA) content were tested by using enzyme-linked immunosorbent assay (ELISA), while peripheral CD62P and GP IIb/IIIa expressions were detected by using flow cytometry before, after, and seven days after RFCA.

RESULTS: D-Dimer and PAI-1 levels increased, while t-PA reduced in group A compared with that in group B and control ($p<0.05$). D-Dimer and t-PA contents gradually elevated, whereas t-PA level gradually declined in group A before, after, and seven days after RFCA ($p<0.05$). Serum CD62P and GP IIb/IIIa expressions in group A were significantly higher compared to that in group B and control ($p<0.05$). CD62P and GP IIb/IIIa levels were significantly higher seven days after RFCA compared with immediate after RFCA in group A ($p<0.05$). CD62P showed a positive correlation with GP IIb/IIIa in hypercoagulable state patients after RFCA ($p<0.05$).

CONCLUSIONS: AF patient may appear in hypercoagulable state after RFCA. CD62P and GP IIb/IIIa significantly increased and exhibited a positive correlation.

Key Words:
CD62P, GP IIb/IIIa, Atrial fibrillation, Radiofrequency catheter ablation, Hypercoagulation.

Introduction

Atrial fibrillation (AF) is a type of arrhythmia with high incidence in clinic that commonly appears in elderly patients. It is showed that elderly patients aged over 80 accounts for 7.5% of the total incidence. AF can be divided into acute and chronic subtypes. Among them, chronic AF is easy to cause thrombosis due to the reduction of atrial contraction ability and blood stasis, which seriously increase the mortality rate. Thus, it needs timely and effective treatment once found[1,2]. As one of the common complications of radiofrequency catheter ablation (RFCA), thrombosis and embolization mostly occur in pulmonary and lower extremity vein with an occurrence of 7%. Following the rapid development of interventional therapy in recent years, RFCA has become the major radical method for AF. It is widely applied in clinic, especially in elderly patients with weak constitutions and multiple chronic diseases. Compared with other therapies, RFCA showed better effectiveness and safety. RFCA can reduce the risk of thrombosis in AF patients. However, as a kind of invasive treatment, RFCA still has the risk of thrombosis in perioperative period. Although anticoagulant drugs are usually used in peri-operative period, such as warfarin and heparin, thrombosis and embolism still cannot be fully

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avoided[3-5]. Platelet microparticle (PMP) refers to the ultramicro membrane vesicles with diameter < 0.5 μm. It is mainly produced in the process of platelet activation and apoptosis[6]. It was suggested that PMP level elevated in patient under hypercoagulable state. PMP not only promotes coagulation, but also participates in inflammation and regulating cell interaction[7]. CD26P belongs to the selectin family. Its α particle fuses with plasma membrane when the platelet is activated, leading to CD62P up-regulation[8]. GP IIb/IIIa is a surface membrane glycoprotein and biomarker of platelet activation. GP IIb/IIIa highly expresses on the surface of platelet and the steric configuration is changed during platelet activation[9]. This study tested platelet particle CD62P and platelet activation biomarker GP IIb/IIIa expressions in AF patients treated by RFCA, and aimed to analyze their relationships with hypercoagulable state after RFCA.

Patients and Methods

Patients

A total of 60 AF patients received RFCA in The Affiliated Hospital of Qingdao University between Jan 2016 and Jun 2017 were enrolled. AF was diagnosed according to the criteria established by WHO[3]. There were 34 males and 26 females with mean age at 38.2 ± 10.1 (18-62) years old. The patients were divided into group A as hypercoagulable state group and group B as non-hypercoagulable group. Group A contained 15 males and 10 females with average age of 34.7 ± 9.3 (18-60) years old. Group B was composed of 17 males and 18 females with mean age of 35.7 ± 8.9 (18-60) years old. Another 60 healthy volunteers were selected as control, including 30 males and 30 females with mean age of 39.1 ± 10.5 (18-65) years old. No apparent abnormality was found in blood routine, urine routine, stool routine, blood pressure, blood glucose, hepatorenal function, cardiac color ultrasound, electrocardiogram, and chest radiography. Treadmill exercise testing was negative. No statistical difference was observed on gender and age among groups (p>0.05).

Inclusion criteria: no thromboembolism or hemorrhagic disease history, no hormone used within the last three months, no ovary or uterus disease, no severe hepatorenal or autoimmune disease, normal blood routine, urine routine, and hepatorenal function. Exclusion criteria: hemorrhagic disease history, cerebral infarction, thrombocytopenia or anti-platelet drug used within two weeks, operation history within three months, uncontrolled severe ventricular arrhythmias and atrioventricular block over degree II without pacemaker, uncontrolled hypertension, diabetes, or hyperlipidemia, uncorrected hypokalemia, digitalism, or electrolyte disturbance, contrast agent allergy, severe renal insufficiency, other RFCA contraindication. This study has been pre-approved by the Ethical Committee of The Affiliated Hospital of Qingdao University. All subjects have signed the informed consent before recruitment in this study.

Reagents and Instruments

Flow cytometry and fluorescence-labeled antiplatelet glycoprotein antibody were derived from Becton-Dickinson (Parsippany, NJ, USA). 0.82 μm standard micro-spheres, PerCP labeled IgG1 mouse antibody and platelet activator were obtained from Sigma-Aldrich (St. Louis, MO, USA). CD62P and GP IIb/IIIa antibodies were purchased from BD Biosciences (San Jose, CA, USA). D-Dimer, parathyroid activity index 1 (PAI-1), and tissue plasminogen activator (t-PA), enzyme-linked immunosorbent assay (ELISA) kits were bought from Sunbio (Shanghai, China). Mouse anti-human CD62P and GP IIb/IIIa polyclonal primary and rabbit anti-mouse secondary antibodies were got from Gibco (Grand Island, NY, USA).

ELISA

The patient received electrophysiological examination for diagnosis. Then, the patients were treated by RFCA by using the 8F catheter through femoral vein or artery. D-Dimer, PAI-1, and t-PA contents were tested before, after, and 7 days after RFCA by using ELISA. The peripheral blood was extracted and the supernatant was centrifuged and stored. The reagent was set at room temperature for 30 min. Next, the standard substance and samples were added to the plate with five replicates. After reacting, washing, developing, and stopping, the plate was read on the micro-plate reader at 450 nm. The equation of linear regression was drawn to calculate the sample concentration.

Flow Cytometry

A total of 3 ml venous blood was extracted in a tube with 3.8% sodium citrate for anticoagulation. After centrifuged at 800 r/min for 30

Table I. Plasma D-Dimer, PAI-1, and t-PA contents. Patients were treated by RFCA by using the 8F catheter through femoral vein or artery. D-Dimer, PAI-1, and t-PA contents were tested before, after, and 7 days after RFCA by ELISA.

Group	D-Dimer (mg/l)	t-PA (μg/l)	PAI-1 (μg/l)
Group A			
Pre-operation	0.24±0.02$^{*\#}$	68.3±7.2$^{*\#}$	59.3±8.2$^{*\#}$
Post-operation	0.54±0.05$^{*\#\&}$	37.4±6.5$^{*\#\&}$	109.2±23.5$^{*\#\&}$
Seven days after operation	0.83±0.08$^{*\#\&@}$	30.3±5.8$^{*\#\&@}$	149.5±26.8$^{*\#\&@}$
Group B			
Pre-operation	0.12±0.01	61.3±6.1	51.8±9.2
Post-operation	0.21±0.02	46.7±7.2	92.4±12.5
Seven days after operation	0.43±0.02	53.8±6.8	115.1±15.7
Control	0.11±0.01	68.4±7.8	50.8±7.5

$^{*}p<0.05$, compared with group B, $^{\#}p<0.05$, compared with control, $^{\&}p<0.05$, compared with pre-operation. $^{@}p<0.05$, compared with post-operation.

min and 3000 r/min for 5 min, the 450 μl plasma containing platelet (3×10^6/μl) were incubated with 20 μmol platelet activator for 5 min at room temperature. A total of 5 μl sample was incubated with antibody at room temperature avoid of light for 15-20 min. Next, the sample was mixed with 1 ml 1% paraformaldehyde at 2-8°C avoid of light for 30 min. The sample was further re-suspended in 1 ml phosphate-buffered saline (PBS) and 1 μl 0.82 μm standard microspheres (1:400). At last, CD62P and GP IIb/IIIa expression rates were tested on flow cytometry by setting FSC, SSC, and FL1-FL3. The data were analyzed by CellQuest software (version 5.1, BD Biosciences, Franklin Lakes, NJ, USA).

Western Blot

The protein was washed and lysed by 50 μl ristocetin-induced platelet aggregation buffer (RIPA) at 0°C. Next, the protein was boiled at 100°C for 5 min. The protein was separated by electrophoresis and transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). After blocked at 4°C overnight, the membrane was incubated in primary antibody (1:100) for 2 h. After washed for four times, the membrane was further incubated in secondary antibody for 1 h. At last, the membrane was developed and analyzed.

Statistical Analysis

The data were analyzed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The measurement data were presented as x ± standard deviation (SD) and compared by using Student's t-test. Enumeration data were compared by χ^2-test. The relationship between CD62P and GP IIb/IIIa was analyzed by Spearman correlation analysis. $p<0.05$ was depicted as statistical significance.

Results

ELISA Detection of Serum D-Dimer, PAI-1, and t-PA Contents

D-Dimer and PAI-I levels increased, while t-PA reduced in group A compared with group B and control ($p<0.05$). D-Dimer and t-PA contents gradually elevated, whereas t-PA level gradually declined in group A before, after, and seven days after RFCA ($p<0.05$) (Table I).

Flow Cytometry Detection of CD62P and GP IIb/IIIa Expressions

Plasma CD62P and GP IIb/IIIa expressions in group A were significantly higher than that in group B and control ($p<0.05$). CD62P and GP IIb/IIIa levels were markedly higher on seven days after RFCA compared with immediate after RFCA in group A ($p<0.05$) (Table II, Figure 1).

Western Blot Detection of CD62P and GP IIb/IIIa Expressions

CD62P and GP IIb/IIIa expressions in group A were significantly higher than that in group B and control ($p<0.05$). CD62P and GP IIb/IIIa levels were markedly higher on seven days after RFCA compared with immediate after RFCA in group A ($p<0.05$) (Table III, Figure 2).

Plasma CD62P and GP IIb/IIIa Expression Correlation Analysis

CD62P showed a positive correlation with GP IIb/IIIa in hypercoagulable state patients after RFCA ($r=0.640$, $p<0.01$).

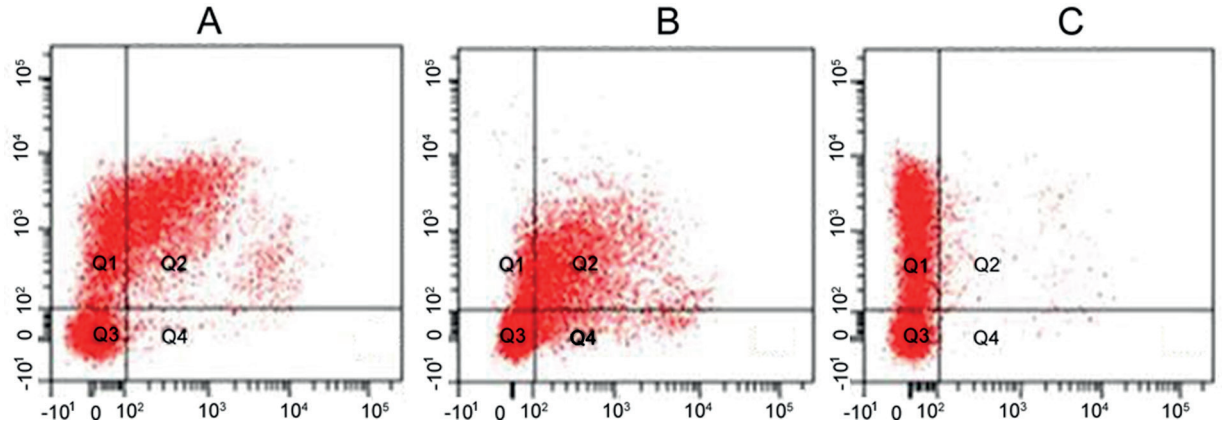

Figure 1. Flow cytometry detection of plasma CD62P and GP IIb/IIIa expressions on the 7th day after RFCA. ***A,*** Group A. ***B,*** Group B. ***C,*** Control.

Table II. Plasma CD62P and GP IIb/IIIa expressions. Patients were treated by RFCA by using the 8F catheter through femoral vein or artery. D-Dimer, PAI-1, and t-PA contents were tested before, after, and 7 days after RFCA by ELISA.

Group	A	B	Control
CD62p			
Pre-operation	3.23±1.01*#	1.76±0.52	1.12±0.34
Post-operation	8.97±1.85*#&	5.43±1.07	4.23±1.12
Seven days after operation	12.33±2.04*#&@	7.31±1.48	5.25±1.81
GP IIb/IIIa			
Pre-operation	6.33±1.02*#	3.41±0.37	2.82±0.11
Post-operation	12.89±2.83*#&	8.07±1.02	7.14±0.51
Seven days after operation	18.61±2.92*#&@	8.83±1.08	7.91±1.07
Oliguria or anuria	2	2	-

*p<0.05, compared with group B, #p<0.05, compared with control, &p<0.05, compared with pre-operation, @p<0.05, compared with post-operation.

Table III. CD62P and GP IIb/IIIa expressions. Protein was isolated followed by measuring the expression of CD62P and GPIIb/IIIa by western blot.

Group	A	B	Control
CD62p			
Pre-operation	0.389±0.004*#	0.322±0.002	0.313±0.005
Post-operation	0.401±0.006*#&	0.315±0.004	0.404±0.003
Seven days after operation	0.578±0.003*#&@	0.428±0.002	0.413±0.003
GP IIb/IIIa			
Pre-operation	0.403±0.004*#	0.347±0.002	0.332±0.002
Post-operation	0.494±0.003*#&	0.375±0.001	0.398±0.001
Seven days after operation	0.625±0.002*#&@	0.402±0.001	0.406±0.003

*p<0.05, compared with group B, #p<0.05, compared with control, &p<0.05, compared with pre-operation, @p<0.05, compared with post-operation.

Discussion

AF is a common arrhythmia in clinic that seriously affects the quality of life. It is easy to cause stroke and heart failure[10]. RFCA, firstly applied in 1994, can rapidly convert and maintain sinus rhythm, and reduce the occurrence of cardiovascular events. It markedly improves the quality of life with better efficacy and safety than internal medicine conservative treatment[11,12]. This study tested platelet particle CD62P and platelet activation biomarker GP IIb/IIIa expressions in AF patients treated by RFCA, aimed to analyze their relationships with hypercoagulation after RFCA.

D-Dimmer is a marker of hypercoagulation. Its fibrous protein can be hydrolyzed by fibrinolytic

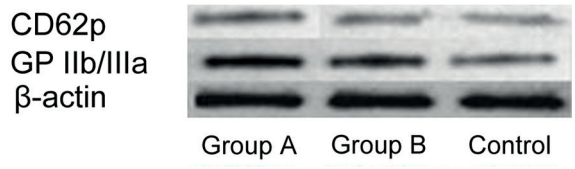

Figure 2. Western blot detection of CD62P and GP IIb/IIIa expressions on the 7th day after RFCA.

enzyme by crosslinking with activation factor, therefore forming high concentrations of degradation products, which shows thrombin generation increase and fibrinolytic activity elevation[13]. t-PA and PAI-1 are sensitive biomarkers to reflect the fibrinolytic activity[14]. In this study, we tested D-Dimer, PAI-1, and t-PA contents in AF patients after RFCA. D-Dimer and PAI-I levels increased. However, t-PA reduced in group A compared with group B and control. D-Dimer and t-PA contents gradually elevated. Meanwhile, the t-PA level gradually declined in group A before, after, and seven days after RFCA. It suggests that RFCA may damage intima, leading to platelet adhesion, aggregation, fibrin formation, and t-PA reduction[15], which may be one of the reasons of hypercoagulation in AF patients after RFCA.

Plasma CD62P and GP IIb/IIIa expressions in group A were significantly higher than that in group B and control ($p<0.05$). CD62P and GP IIb/IIIa levels were significantly higher seven days after RFCA compared to immediate after RFCA in group A. CD62P is a member of selectin family that can specifically reflect the activation of platelet, thus to be treated as the golden standard of platelet activation[16,17]. Multiple media can activate platelet to form GP IIb/IIIa receptor[18,19]. GP Ib exposed by platelet after activation binds to vwF. GP IIb/IIIa receptor binds to fibrinogen. Intima injury more easily causes platelet aggregation and thrombosis[20,21]. Our results indicated that hypercoagulation AF patients after RFCA suffered from high levels of CD62P and GP IIb/IIIa before the operation. CD62P and GP IIb/IIIa expressions increased after RFCA, revealing platelet aggregation and activation after RFCA. It is easy to cause atherosclerotic plaque formation, resulting in platelet activation maintenance.

At last, this work analyzed the relationship between serum CD62P and GP IIb/IIIa after RFCA. CD62P showed a positive correlation with GP IIb/IIIa in hypercoagulable state patients. Traditional anti-platelet drug mainly inhibits thromboxane A2 and adenosine diphosphate mediated platelet activation instead of aggregation[22]. This study demonstrated that GP IIb/IIIa expression was positively correlated with platelet activation. Reasonable application of GP IIb/IIIa receptor inhibitor may suppress platelet aggregation and reduce the risk of thrombosis.

Conclusions

We showed that D-Dimer and PAI-I levels increased, while t-PA reduced in AF patients treated by RFCA. CD62P and GP IIb/IIIa levels were high in hypercoagulation patients before operation. Their expressions markedly elevated after RFCA and exhibited positive correlation. CD62P and GP IIb/IIIa monitoring should be widely promoted in clinic. Better therapeutic schedule based on the condition and detection results may improve the prognosis. The pathogenesis of AF involves multiple cytokines. Their interactions with signaling pathway were complex. Precise detection of CD62P and GP IIb/IIIa expressions may provide a new strategy for the treatment of AF.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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