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Platelet function and microparticle levels in atrial fibrillation: Changes during the acute episode

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ABSTRACT

Background: Thrombotic risk constitutes a major complication of atrial fibrillation (AF). Platelets and microparticles (MPs) are important for hemostasis and thrombosis, however their participation during AF is not well known. The aim of this study was to characterize platelet function and MPs procoagulant and fibrinolytic activity in AF patients and to determine the effects of an acute-AF episode.
Methods: Blood was collected from paroxysmal (21) and persistent (16) AF patients referred for AF catheter ablation. Ten patients in sinus rhythm for 10 days were induced in AF allowing comparisons of left atrium samples before and after induction. Platelet aggregation with ADP, TRAP, collagen, and ristocetin was studied. Platelet surface expression of PAR-1, αIIbβ3, GPIb and P-selectin were evaluated by flow cytometry, and MPs-associated procoagulant and fibrinolytic activity levels were determined by functional assays.
Results: A specific reduction in platelet aggregation to TRAP, activating the thrombin receptor PAR-1, was found in all AF patients. No differences in platelet receptor expression were found. Yet, after acute-induced AF, the platelet response was improved. Furthermore, a significant decrease of left atrium tissue factor-dependent procoagulant activity of MPs was observed.
Conclusion: Acute episodes of AF results in a decrease in MPs-associated tissue factor activity, possibly corresponding to consumption, which in turn favors coagulation and the local production of thrombin. A decreased platelet basal aggregation to TRAP may result from PAR1 desensitization, whereas the improved response after an induced episode of AF suggests activation of coagulation and PAR1 re-sensitization.

1. Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia, occurring in 1–2% of the world's population with risk increasing with age [1]. Radiofrequency ablation emerged in the late 1990's, and is now an important approach in treating AF decreasing the burden of the arrhythmia [2,3]. One of the main complications of paroxysmal or permanent forms of AF is increased risk of a stroke; with 20% of stroke patients being related to AF [1,4]. Vitamin-K antagonist (VKA), and more recently direct oral anticoagulants (DOAC) are a cornerstone of anti-thrombotic treatments for patients with AF. Nevertheless, even with these drugs, stroke may still occur in patients presenting AF.

Abbreviations: ADP, adenosine diphosphate; AF, atrial fibrillation; DOAC, direct oral anti-coagulant; MPs, microparticles; NSAIDs, nonsteroidal anti-inflammatory drugs; TF, tissue factor; TRAP, thrombin receptor activating peptide; VKA, vitamin-K antagonist; VWF, von Willebrand factor.

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Thromboembolic risk is linked to different pathophysiological mechanisms, including hemodynamic dysfunction and modifications of hemostatic parameters. Hemodynamic problems are most often due to modifications of blood flow in the left atrium (with the loss of atrial contraction and atrio-ventricular synchronization), atrial tissue remodeling and inflammation processes [5]. Among hemostatic parameters, platelets are important players. They are small, anucleate blood cells that upon endothelial damage form a hemostatic plug, helping to prevent blood loss at sites of vascular injury. They adhere to sites of vascular injury through interactions between glycoprotein (GP) receptors with von Willebrand factor (VWF) and collagen, present in the exposed subendothelial matrix. Platelets then aggregate and secrete biologically active substances, proteins and newly synthesized active metabolites [6]. They form a procoagulant surface enhancing the coagulation cascade ending in the formation and stabilization of the hemostatic plug favoring thrombin generation and fibrin formation. Secreted proteins are capable of influencing processes such as angiogenesis, inflammation and the immune response. During activation or apoptotic processes, large numbers of small vesicles called microvesicles or microparticles (MPs) may form and be shed from the surface of the platelets, leukocytes and vascular cells [7]. MPs are important in thrombosis, inflammation and vascular reactivity. They behave as biological drones playing a key role in the fine-tuning of vascular homeostasis. Beyond their well-described procoagulant properties, accumulating data has shown that specific endothelial cell or leukocyte-derived MPs bind plasminogen and vectorize plasminogen activators, leading to an efficient plasmin generation and matrix metalloproteinases activation [8]. Studies with AF patients did not show increased plasmin activity, but an abnormal fibrinolytic balance [9]. If AF is associated with an increased thrombotic tendency the role of platelets and other changes needs to be established.

We have analyzed in non-valvular AF, the platelet aggregation response, expression of selected platelet membrane GPs, and the levels of MPs-linked procoagulant and fibrinolytic activities. These parameters were measured in paroxysmal and persistent AF patients from peripheral blood samples and directly from blood in the left atrium during the ablation procedure. As atrial fibrillation had to be induced in some patients to map atrial fragmented signals, this procedure allowed us to examine if the newly identified biomarkers were directly linked to an acute episode of fibrillation.

2. Methods

2.1. Patients

Paroxysmal and persistent AF patients referred for primary AF catheter ablation were included in this study and baseline characteristics are described in Table 1. Paroxysmal AF patients ($n = 21$) were defined as having at least 2 self-converting episodes lasting <7 days. Persistent AF patients ($n = 16$) were defined as AF lasting longer than 7 days [3]. Healthy control subjects ($n = 11$) were from the hospital and research center staff. Exclusion criteria were active smokers, antiplatelet treatment, history of acute cardiac events in the preceding 3 months, valvular heart diseases, chronic inflammatory diseases, chronic renal and hepatic disease, and uncontrolled hypertension. In our 37 patient cohort, 24 patients were administered with 100 mg of ketoprofen (nonsteroidal anti-inflammatory drugs (NSAIDs)) before the beginning of the procedure and heparin was given after the trans-septal puncture.

This study was in accordance with the declaration of Helsinki and was approved by the local institutional review committee (2016-A00603-48). Informed consent was signed by the patients and controls.

2.2. Blood collection

Blood samples from AF patients were taken during AF ablation procedures, using a trans-septal approach [3]. Citrated and EDTA blood samples were collected from the femoral vein, and the left atrium immediately after trans-septal puncture and heparin administration. Among the 37 patients included in the study, a subgroup of patients in sinus rhythm ($n = 10$) throughout the 10 days prior to ablation were subjected to induced-AF by pacing at the coronary sinus catheter (paroxysmal AF $n = 5$, persistent AF $n = 5$). After 20 min of continuous AF, additional blood samples were collected from the left atrium (before radiofrequency energy delivery). Peripheral blood samples were collected from healthy volunteers via the cubital vein.

Table 1
Baseline characteristics of AF patients.

	Paroxysmal AF ($n = 21$)	Persistent AF ($n = 16$)	p value
Age	60 ± 2.6	59 ± 2.0	$p = 0.63$
Men/women	14:7	14:2	$p = 0.25$
BMI	26.3 ± 1.0	26.6 ± 1.0	$p = 0.84$
CHA2DS2-VASc Score	1.3 ± 0.2	0.9 ± 0.3	$p = 0.44$
Duration of AF (years)	6 ± 1.2	5.8 ± 1.1	$p = 0.90$
Comorbidities			
Hypertension (%)	28.6	18.8	$p = 0.70$
Diabetes mellitus (%)	4.8	0	$p = 1.00$
Stroke/transient ischemic attack (%)	9.5	0	$p = 0.50$
Treatment			
Class I antiarrhythmics (%)	57.1	25	$p = 0.05$
Class II antiarrhythmics (%)	14.3	43.8	$p = 0.07$
Class III antiarrhythmics (%)	33.3	81.3	$^{**}p = 0.01^{**}$
VKA (%)	47.6	25.0	$p = 0.19$
DOAC (%)	42.9	75.0	$p = 0.09$
Echocardiographic parameters			
LA surface (cm^2)	19.6 ± 0.9	24.5 ± 2.9	$p = 0.12$
LVEF (%)	62.1 ± 1.4	58.3 ± 4.5	$p = 0.92$

Value are mean ± SEM or %. $^{**}p \leq 0.01$. AF = atrial fibrillation; BMI = body mass index; CHA2DS2-VASc Score = congestive heart failure, hypertension, age ≥ 75 years, diabetes, stroke, vascular disease, age 65 to 75 years, sex category (female); LA = left atrium; LVEF = left ventricular ejection fraction, DOAC = direct oral anti-coagulant; VKA = vitamin K antagonist.

2.3. Platelet function

Blood cell counts were recorded. Platelet-rich plasma (PRP) and platelet-poor plasma were prepared following the SSC/ISTH recommendations. PRP platelet count lower than $150 \times 10^{\wedge 9}\ l^{-1}$ and higher than $600 \times 10^{\wedge 9}\ l^{-1}$ were excluded [10]. Platelet aggregation was assessed using an APACT4004 aggregometer (Elitech, Puteaux, France) by light transmission over 10 min after adding adenosine diphosphate (ADP) 10 μM, ristocetin 1.5 mg/ml (Elitech, Puteaux, France), Horm collagen 2 μg/ml (Stago, Taverny, France) or thrombin receptor activating peptide (TRAP-6mer) 10 μM (Bachem, Bubendorf, Switzerland). Percentage of maximal intensity aggregation and maximal disaggregation after addition of agonists were determined from the recordings [9].

2.4. Platelet membrane receptor expression

Expression of αIIbβ3 and GPIb was evaluated by flow cytometry with a FacsCanto I (BD biosciences, Franklin Lakes, New Jersey, USA) as previously described [11]. For PAR-1 and P-selectin detection, mouse anti-thrombin receptor antibody PE conjugates (clones WEDE15 and SPAN12) (Beckman-Coulter, Brea, California, USA) and mouse anti-CD62P-PE (BD, biosciences, Franklin Lakes, New Jersey, USA) were mixed with 10^6 platelets. Negative controls consisted of the addition of isotype mouse IgG1-PE antibodies (Beckman Coulter, Brea, California, USA and BD Biosciences, Franklin Lakes, New Jersey, USA). Levels of αIIbβ3, GPIb and PAR-1 were determined as mean fluorescence intensity (MFI) and the level of P-selectin expression was determined as a percentage of positive cells compared to the negative control set at 1%.

2.5. Evaluation of microparticle functions

Platelet free plasma samples were prepared following the SSC/ISTH recommendations [12]. TF-dependent procoagulant activity of MPs was determined using a fluorogenic assay of factor Xa generated by purified MPs as described in Agouti et al. [13]. The fibrinolytic activity of MPs was determined using a chromogenic test of plasmin generated by purified MPs as modified by BioCytex (separation of plasma MPs by immuno-magnetic separation) [14].

2.6. Statistical analysis

Categorical variables were compared using Fisher's exact or Pearson's chi-square test as deemed appropriate. As normality or equal variance test failed on several continuous variables and the small size of the study, statistical analysis was performed by non-parametric tests. To compare the control, paroxysmal and persistent AF groups for platelet function, membrane receptor expression and MPs, the Kruskall-Wallis test was performed. A Mann-Whitney test was used to determine the effect of NSAID administration and cardiac rhythm during blood sampling on maximal aggregation variables. The effect of an acute AF episode and site sampling on platelet function, membrane receptor expression and MPs was analyzed by Wilcoxon test. Statistical significance was established at $p < 0.05$. All data was analyzed using GraphPad Prism (version 6.07).