Thrombosis Research 136 (2015) 408–414

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Denser plasma clot formation and impaired fibrinolysis in paroxysmal and persistent atrial fibrillation while on sinus rhythm: Association with thrombin generation, endothelial injury and platelet activation

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ARTICLE INFO

Article history:
Received 10 February 2015
Received in revised form 4 May 2015
Accepted 25 May 2015
Available online 29 May 2015

Keywords:
Atrial fibrillation
Clot lysis time
Fibrinolysis
Thrombin
Thromboembolism

ABSTRACT

Introduction: Formation of compact and poorly lysable fibrin clots have been demonstrated in patients following ischemic stroke. Recently, it has been shown that denser fibrin networks and impaired fibrinolysis occurs in subjects with permanent atrial fibrillation (AF). Fibrin clot phenotype in other types of AF remains to be established. We evaluated fibrin clot properties in paroxysmal (PAF) and persistent AF (PsAF).

Material and Methods: We studied 88 non-anticoagulated patients with AF on sinus rhythm and free of stroke (41 with PAF, 47 with PsAF) versus 50 controls. Ex-vivo plasma fibrin clot permeability (K_s) and clot lysis time (CLT) were evaluated along with von Willebrand factor (vWF), peak thrombin generation (TG), platelet factor 4 (PF4) and fibrinolytic proteins.

Results: Compared with control subjects, clots obtained from plasma of patients with PAF and PsAF had similarly lower K_s (−7.7%, P = 0.01; −8.6%, P = 0.005, respectively) and prolonged CLT (+10.8%, P = 0.006; +7.8% P = 0.04, respectively). No associations of K_s and CLT with CHA_2DS_2-VASc and HAS-BLED score were observed. Patients with AF had higher TG, vWF, PF4 and plasminogen activator inhibitor-1 (PAI-1) antigen compared with controls. Multiple linear regression adjusted for age, gender, body mass index and fibrinogen showed that TG ($\beta = -0.41$), vWF ($\beta = -0.29$) and PF4 ($\beta = -0.28$) are the independent predictors of K_s ($R^2 = 0.78$), while CLT was independently predicted by TG ($\beta = 0.37$), PAI-1 antigen ($\beta = 0.29$) and vWF ($\beta = 0.26$) in the AF group ($R^2 = 0.39$).

Conclusions: Patients with PAF and PsAF while on sinus rhythm display unfavorably altered fibrin clot properties, which might contribute to thromboembolic complications.

1. Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia that is associated with an increased risk of stroke and thromboembolic complications. Paroxysmal AF (PAF), persistent AF (PsAF) and permanent AF, all significantly increase the stroke risk up to 20% annually [1]. The major risk factors for stroke in AF include cardiac failure, hypertension, age, diabetes, and previous stroke/transient ischemic attack/thromboembolism [2]. Prothrombotic mechanisms underlying AF–related thromboembolism are multiple and include impaired blood rheology, endothelial dysfunction, blood coagulation activation, platelet activation, and altered fibrinolytic activity [3–5]. Elevated plasma fibrinogen, von Willebrand factor (vWF), and soluble P-selectin have been shown to be linked to thromboembolic events in AF patients [6,7]. An increase in thrombin generation (TG) in circulating venous blood is observed during or shortly after PAF episodes [8]. Patients with permanent AF and stroke or AF alone present higher TG than those on sinus rhythm without stroke [9]. TG measured in blood obtained from the left atrium (LA) is similarly elevated in patients with PAF in the non-paroxysmal period and PsAF [10].

Available data on fibrinolysis in AF patients are sparse. It has been reported that patients with PAF and PsAF exhibit higher levels of tissue plasminogen activator antigen (t-PA), which has been demonstrated to predict adverse cardiovascular events and all-cause death in patients with AF [11]. Elevated t-PA antigen is considered a marker of endothelial dysfunction and vascular injury in this disease [12], or reflects increased formation of inactive t-PA/PAI-1 complexes, indicating elevated plasminogen activator inhibitor-1 antigen (PAI-1), a major inhibitor of fibrinolysis [13,14]. There have been reports showing increased plasma plasmin-antiplasmin complexes in PAF and PsAF patients compared with control subjects, suggesting a hyperfibrinolytic state [15,16], although high D-dimer concentrations reflect largely enhanced thrombin

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http://dx.doi.org/10.1016/j.thromres.2015.05.028
0049-3848/

and fibrin formation. Interestingly, it has been shown that in patients with permanent AF and previous ischemic stroke, there is impaired fibrin clot lysis using a well-established global lysis test and this phenomenon shows association with elevated plasma levels of PAI-1, thrombin-activatable fibrinolysis inhibitor antigen (TAFI), α_2-antiplasmin (α_2AP) and soluble thrombomodulin [17]. Very recently, Zabczyk et al. have reported that long-term treatment with vitamin K antagonists (VKA) in PsAF patients results in formation of permeable clots susceptible to fibrinolysis [18]. To our knowledge, fibrin clot phenotype in other types of AF remains to be established.

Compelling evidence indicates that impaired fibrinolysis results in part from a tendency to form more compact plasma clots as evidenced by low clot permeability [19,20]. Altered fibrin clot properties have been reported in acute or previous ischemic stroke [21–23], venous thromboembolism (VTE) [24], and atherosclerotic vascular disease, in particular myocardial infarction (MI) [25,26]. Moreover, formation of a compact fibrin clot has been shown in patients with diabetes [27], arterial hypertension [28] and heart failure [29], which represent the well-established risk factors for stroke in AF patients [30]. To our knowledge, there have been no reports on fibrin clot properties in PAF and PsAF patients on sinus rhythm, who did not experience stroke or any other thromboembolic episodes, that by themselves could unfavorably alter clot characteristics.

The aim of this study was to evaluate the plasma clot properties and their determinants in patients with paroxysmal and persistent AF. We hypothesized that in such patients even on sinus rhythm thromboembolic risk is related to formation of compact plasma fibrin clots resistant to plasmin-mediated lysis.

2. Methods

2.1. Patients

We studied 88 consecutive patients with documented nonvalvular AF, according to current practice guidelines, who at the time of enrollment were on sinus rhythm [2]. PAF was diagnosed when episodes were self-terminating and continuing for up to 7 days, while PsAF was defined as AF episodes lasting longer than 7 days or requiring termination by cardioversion. The AF patients were eligible if since the last documented or self-reported AF episode elapsed at least 1 month. All control subjects underwent full clinical examination and 12 lead ECG was performed to exclude atrial fibrillation. None of the controls had a history of arrhythmia.

The exclusion criteria were acute coronary syndrome within the preceding 12 months, significant valvular heart disease, congenital heart disease, chronic kidney disease stage 3 or more, hepatic injury (alanine aminotransferase [ALT] 1.5 times above the upper limit of normal), known malignancy, signs of acute infection, history of autoimmune diseases, C-reactive protein (CRP) > 10 mg/l, history of documented VTE, stroke or transient ischemic attacks. VKA were stopped and replaced by enoxaparin (1 mg/kg twice daily) 10–14 days before the blood draw.

Age-, gender- and body mass index-matched (BMI) asymptomatic subjects without history of AF (n = 50), who were recruited at an outpatient clinic, served as controls.

Data were collected using a standardized questionnaire, which contained demographic characteristics and information about cardiovascular risk factors, comorbidities and treatment.

Arterial hypertension was diagnosed based on a history of hypertension (blood pressure > 140/90 mmHg) or preadmission antihypertensive treatment. Hypercholesterolemia was defined as serum total cholesterol over 5.2 mmol/l. Diabetes mellitus was defined as fasting glucose ≥7.0 mmol/l on two separate occasions or use of insulin or oral antidiabetic agents. HbA1c was not measured in all study participants. Most of them had HbA1c ≤7%. The BMI was defined as body weight divided by the square of the height in meters (kg/m^2). Coronary artery disease was defined as the presence of angina pectoris or myocardial infarction or documented history of any revascularization of the coronary arteries. Heart failure was defined as the presence of relevant symptoms and signs and left ventricular ejection fraction ≤45% [30].

The CHA_2DS_2-VASc (Congestive heart failure/left ventricle dysfunction, Hypertension, Age ≥75 years, Diabetes mellitus, previous Stroke/transient ischemic attack/thromboembolism, Vascular disease, Age 65–74 years, Sex category) and $CHADS_2$ (Cardiac failure, Hypertension, Age, Diabetes, Stroke) scores were used to assess the risk for stroke and thromboembolism in AF patients. Scores of 0, 1 and ≥2 were defined as the low, intermediate and high thromboembolic risk categories, respectively, for each index [31,32]. The HAS-BLED (Hypertension, Abnormal renal/liver function, Stroke, Bleeding history or predisposition, Labile INR, Elderly > 65, Drugs/alcohol concomitantly) score was used to assess the risk of bleeding. Scores of 0–2 and ≥3 were defined as the low and high bleeding risk, respectively [33].

The study was approved by the Ethical Committee of the Jagiellonian University. All participants gave informed consent.

2.2. Laboratory Investigations

Fasting venous blood was drawn from an antecubital vein with minimal stasis between 8 and 11 AM. The blood samples were mixed with 3.2% sodium citrate (9:1), centrifuged within 20 min at 1500 g. Supernatant was aliquoted and stored at −80 °C until analysis.

Complete blood count, lipid profiles, glucose, creatinine, ALT were assayed by standard laboratory methods. Fibrinogen concentrations were determined using the von Clauss method. High-sensitivity CRP levels were measured by latex nephelometry (Siemens, Marburg, Germany). Commercially available ELISAs were used to determine in citrated plasma t-PA antigen (Diagnostica Stago, Asnieres, France), PAI-1 antigen (American Diagnostica, Stamford, CT, USA), α_2-antiplasmin and plasminogen (STA Stachrom α2-antiplasmin and STA Stachrom plasminogen, Diagnostica Stago, Asnieres, France), vWF antigen (Diagnostica Stago, Asnieres, France), and PF4 (R&D Systems, Minneapolis, MN, USA). Peak thrombin generation was measured in plasma of circulating venous blood using the calibrated automated thrombogram as previously described [34].

All measurements were performed by technicians blinded to the sample status. The coefficients of interassay variations for fibrin variables and ELISAs were <7%.

2.3. Fibrin Clot Permeability

Fibrin clot permeability was measured as described previously [35]. Briefly, plasma was mixed 20 mM $CaCl_2$ and 1 U/ml human thrombin (Sigma-Aldrich, St Louis, MO, USA). Tubes containing the clots were connected via plastic tubing to a reservoir of a buffer (0.05 M Tris–HCl, 0.15 M NaCl, pH 7.5). The volume flowing through the gels was measured within 60 min. Permeation coefficient (K_s), which indicates the pore size was calculated from equation: $K_s = Q \times L\ \eta/t \times A \times \Delta p$, where Q is the flow rate in time t, L is the length of a fibrin gel, η is the viscosity of liquid, A is the cross-sectional area and Δp is a differential pressure in dyne cm^{-2}. The intraindividual variability of results was 7%.

2.4. Clot Lysis Assay

Clot lysis time was measured using a tissue factor (TF)-induced clot lysis assay as described previously [36,37]. Briefly, citrated plasma was mixed with 15 mM $CaCl_2$, 10000-diluted human tissue factor (Innovin, Siemens, Marburg, Germany), 12 μM phospholipid vesicles (Avanti PolarLipids, Alabaster, AL, USA), and 60 ng/ml recombinant tPA (Boerhinger Ingelheim, Ingelheim, Germany). Turbidity was measured at 405 nm at 37 °C. CLT was defined as the time from the midpoint of