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RESEARCH ARTICLE

THROMBIN ACTIVATABLE FIBRINOLYSIS INHIBITOR IN THE EARLY HOURS OF PAROXYSMAL ATRIAL FIBRILLATION

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ABSTRACT

Introduction: There is evidence that hemostatic disorders occur very early after clinical manifestations of paroxysmal atrial fibrillation (PAF). **Aim:** To investigate the activity of thrombin activatable fibrinolysis inhibitor (TAFI) before the twenty-fourth hour of the occurrence of PAF, given the important role it plays in maintaining hemostatic balance. **Materials and method:** 51 non-anticoagulated patients were studied (26 men, 25 women; mean age 59.84±1.60 years) with PAF duration <24 hours and 52 controls (26 men, 26 women; mean age 59.50±1.46) without data on the manifestation of the disease to date. TAFI activity in plasma was measured by colorimetric assay (Stachrom, TAFI, Diagnostica Stago, France). **Results:** In PAF group TAFI activity was significantly lower compared to that of the control group in sinus rhythm (62.70±4.71 vs 115.6±4.02%, p <0.001). **Conclusion:** TAFI activity is significantly reduced in the first twenty-four hours of the clinical presentation of PAF. These early changes suggest their close relationship with the disease. At the same time they are a prerequisite for an increased activity of the fibrinolytic system in plasma, which may explain the low embologenic potential during the first hours of PAF.

INTRODUCTION

Over the past two decades atrial fibrillation (AF) is increasingly defined as the "new epidemic", given its increasing morbidity (Lip *et al.*, 2007). The clinical manifestation of AF is associated with an increased risk of thromboembolic events, making it a disease with social value (Zoni-Berisso *et al.*, 2014). Epidemiological studies show that approximately 20% of all strokes develop in conditions of AF and this percentage is greater than 25 in the population over 85 years. Patients with AF have a five times higher age-adjusted risk of stroke than the population without the disease (Kirchhof *et al.*, 2007; Wolf at al., 1991).

The risk of thromboembolic events in AF is determined to a significant extent by the duration from the occurrence of the disease. It is well known that the early manifestation of the arrhythmia (according to some authors up to 24 hours, while to other up to 48 hours) has low embologenic potential (Camm *et al.*, 2010). This fact raises the clinical and research interest in the hemostatic profile in the first hours of the disease.

In this regard a study was conducted on patients with paroxysmal atrial fibrillation (PAF). The groups, however, were extremely heterogeneous with respect to the duration from the occurrence of the episode, namely from a few hours to 7 days. There are no studies entirely directed towards the first hours (up to 24th hour) of the disease. Furthermore, studies are few and the results are not unidirectional. For example, the activity of the fibrinolytic system, as well as the process of thrombogenesis, is represented as significantly increased to significantly reduced (Marin *et al.*, 2004; Lip *et al.*, 1996; Feinberg *et al.*, 1999; Drabik *et al*, 2015; Freynhofer *et al.*, 2013). Controversial are also the data on endothelial damage/dysfunction and platelet activation (Li-Saw-Hee *et al.*, 2001; Hatzinikolaou-Kotsakou *et al.*, 2003). The lack of uniform results is a reason for studies to continue.

It is well known that TAFI is one of the main components of the hemostatic system (Dobrovolsky, 2002). It is defined as a fibrinolysis inhibitor and at the same time as one of the main intermediates between the coagulation and fibrinolytic system. TAFI plays an important role in the cross-regulation of these systems and respectively in preserving haemostatic balance (Mosnier *et al*, 2006). According to literature it is directly

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related to the formation of thromboembolic potential (Gorog, 2010). The above-mentioned facts were a prerequisite for conducting this study.

Aim: To investigate TAFI activity before the twenty fourth hour of the occurrence of PAF, given the important role it plays in maintaining hemostatic balance.

MATERIALS AND METHODS

Study design, study population

Patients with PAF duration <24 hours were screened, who were hospitalized in the Cardiac Intensive Care Unit of the First Cardiology Clinic at the University Hospital St. Marina - Varna for the period October 2010 – May 2012. The duration from the occurrence of the disease was determined based on detailed medical history in which patients reported a sudden onset of "palpitations" continuing until hospitalization. AF diagnosis was accepted after electrocardiographic study done immediately on admittance to the ward. From a total of 338 screened patients, only 51 patients (26 men, 25 women; mean age 59.84±1.60 years) were selected because of the exclusion criteria *(see below)*.

The control group was formed only by volunteers with no previous history and electrocardiographic data for PAF. From a total of 169 screened patients, 52 controls were selected (26 men, 26 women; mean age 59.50±1.46).

We used identical exclusion criteria for the selection of the patient and control group *(see below)*.

The selection of the study participants (patients and controls) aimed at the utmost degree to eliminate or equalize the factors that could affect hemostasis between the two groups.

Exclusion criteria

1. Cardiovascular diseases: ischemic heart disease, heart failure, uncontrolled hypertension, inflammatory diseases of the heart, congenital and acquired heart disease, cardiomyopathies.
2. Other diseases – kidney, lung or liver failure; diseases of the central nervous system; inflammatory and/or infectious diseases in the past three months; neoplastic or autoimmune diseases; diseases of the endocrine system (with the exception of type 2 diabetes mellitus, non-insulin dependent, well-controlled).
3. Intake of hormone replacement therapy or oral contraceptives, pregnancy, systemic administration of analgesics (including NSAIDs), administration of antiplatelet agents and anticoagulants, obesity with BMI>35.
4. Inability to determine the onset of the arrhythmia (used only in patients).

The health status of study participants was determined on the basis of medical history, medical records, physical examination, laboratory tests and multiple electrocardiograms and transthoracic echocardiography.

The study was conducted after approval by the Ethics Committee of Research (№35/29.10.2010) at the the aforementioned hospital and in accordance with the Declaration of Helsinki (WMA declaration of Helsinki, 2008). All participants were included in the study after previously signing an informed consent to participate.

Blood collection and laboratory analysis

We took a blood sample once (3.5 ml venous blood from antecubital vein) from each participant to study TAFI activity in plasma. The blood was collected into coagulation tubes with 3.2% sodium citrate (VACUETTE, Greinet Bio-One North America, Inc.), and then centrifuged at 2500g for 15 min. The plasma was separated and stored at -20°C for up to one month. Re-freezing of samples was not allowed.

TAFI activity in plasma was determined by colorimetric assay (Stachrom, TAFI, Diagnostica Stago, France). The intra-assay coefficient of variation was 4.8%, while the inter-assay was 5.4%.

TAFI activity was established twice in each sample, taking the average of the two measurements for the study.

Statistical analysis

Statistical processing of the results was done using the software GraphPad Prism, Version 5.00, which allowed by descriptive statistics to calculate the mean values, SEM, relative shares and central tendency. Testing the equality of means and indicators' relative share hypotheses was done by Student's t-test. The results were presented as mean±standard error of the arithmetic mean ($\bar{x}$±SEM) or n (%).

RESULTS

Participants' clinical characteristics

There was no statistically significant difference between the patient and control groups in terms of mean age, gender structure, accompanying diseases, deleterious habits and body mass index (BMI) (Table 1).

There were also no significant differences in the indicators measured by standard transthoracic echocardiography (Table 2) ($p > 0.05$).

The statistical analysis indicated that the patients were admitted to the ward between the second and the twenty-fourth hour after the onset of the arrhythmia, most common – during the fifth hour (Mo=5; 10 of all 51 patients). The mean duration of AF episodes until hospitalization was 8.14 ± 0.76 hours.

TAFI activity

TAFI plasma activity in the patient group was significantly

lower compared to that of the control group in sinus rhythm (62.70±4.71 vs 115.6±4.02%, p <0.001; Figure 1).

Table 1 Clinical characteristics of the patient and control group

	Patients with PAF	Control group	P values
Number of participants in the group	51	52	p=0.89
Mean age (years)	59.84±1.60	59.50±1.46	p=0.87
Men/Women	26/25	26/26	p=1/p=0.93
Accompanying diseases			
Hypertension	37 (72.54%)	34 (65.38%)	p=0.44
Diabetes mellitus type 2	3 (5.88%)	2 (3.84%)	p=0.62
Chronic ulcer disease	2 (3.92%)	0	p=0.15
Status after hysterectomy	2 (3.92%)	1 (1.92%)	p=0.54
Benign prostatic hyperthrophy	1 (1.96%)	0	p=0.32
Dyslipidemia	4 (7.84%)	3 (5.77%)	p=0.69
Medicaments for Hypertension and Dyslipidemia			
Beta blockers	19 (37.25%)	17 (32.69%)	p=0.62
ACE inhibitors	15 (29.41%)	14 (26.92%)	p=0.78
Sartans	11 (21.57%)	9 (17.31%)	p=0.58
Statins	4 (7.84%)	3 (5.77%)	p=0.69
Deleterious habits			
Smoking*	8(15.69%)	7(13.46%)	p=0.75
Alcohol intake**	7(13.72%)	6(11.53%)	p=0.74
BMI (kg/m^2)	23.85±0.46	24.95±0.45	p=0.09

*up to 10 cigarettes a week. Hospitalized patients did not smoke for at least 48 hours before the onset of the arrhythmia. Controls were studied after a 48-hour non-smoking period.
**1-2 drinks/week. Hospitalized patients did not drink alcohol for at least 48 hours before the arrhythmia. Controls were studied after a 48-hour period without alcohol.

Table 2 Echocardiographic evaluation of patient and control group

	Patients with PAF	Control group	P values
Echocardiographic indicators			
LVEDD (mm)	52.57±0.58	52.29±0.57	p=0.73
LVESD (mm)	34.43±0.56	34.73±0.48	p=0.69
EF (%)	62.98±0.70	61.54±0.58	p=0.12
IVS (mm)	10.37±0.23	9.92±0.26	p=0.20
PW (mm)	10.24±0.21	9.73±0.28	p=0.16
LA volume (ml/m²)	22.81±0.45	23.82±0.48	p=0.13
RVEDD (mm)	30.54±1.58	29.17±1.52	p=0.18

LVEDD – left ventricular end-diastolic diameter; LVESD – left ventricular end-systolic diameter; EF – ejection fraction; IVS – interventricular septum; PW – posterior wall; LA – left atrium; RVEDD – right ventricular end-diastolic volume

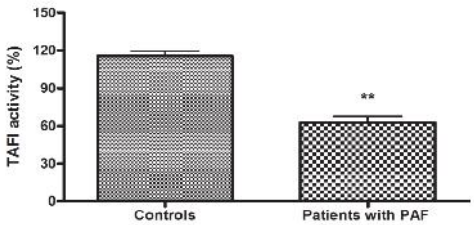

Figure 1 TAFI activity in plasma of patients with PAF and controls in sinus rhythm (** - p<0.001).

DISCUSSION

The hemostatic system is extremely conservative and strictly regulated system that allows the body to (1) restore the integrity of damaged vascular walls, (2) keep the fluidity of blood and (3) remove blood clots (Versteeg *et al*., 2013). Its adequate functioning is the result of a well-regulated platelet activation, coagulation and fibrinolysis. The fine balance between them requires the involvement of multiple vascular, platelet and plasma factors (Tanaka *et al*., 2009). One of these factors is TAFI. It performs the complex role of an intersection as well as connecting point of the processes of coagulation and fibrinolysis (Dubis J, Witkiewicz W, 2010). TAFI deserves special attention also because besides being a hemostatic regulator, it participates in the process of inflammation by inactivating inflammatory mediators such as C3a and C5a, bradykinin, etc. (Campbell *et al*., 2002).

TAFI is a glycoprotein synthesized in the liver, which circulates in the blood as an inactive proenzyme (Bajzar *et al*., 1995). The activated form of TAFI (TAFIa) exhibits anti-fibrinolytic activity by splitting off lysine residues from carboxy-terminals of partially degraded fibrin (Dubis J, Witkiewicz W, 2010). Thus TAFI reduces t-PA and plasminogen binding on the fibrin surface and respectively reduces the formation of plasmin, and fibrin clot is protected from lysis. Therefore TAFI in fact controls fibrinolysis through modulating the fibrin co-factor function in plasminogen activation and not through direct irreversible inhibition. There is evidence also that TAFI exhibits a clot-stabilizing effect by extending the time of t-PA induced lysis (Bajzar *et al*., 1996).

TAFI is activated by the action of a number of biomolecules such as thrombin, trypsin, kallikrein and others. Of these, the most effective is thrombin, alone or within the thrombin-thrombomodulin complex (Chapin JC, Hajjar KA, 2015). As it is well-known thrombin is the main enzyme that catalyzes the conversion of fibrinogen to fibrin in the formation of blood clot in the coagulation system and also, not less importantly, regulates the coagulation system while providing feedback inhibition (Bogatcheva *et al*., 2002). TAFI activity is equally important for both coagulation and fibrinolysis (Mosnier *et al*., 2001).

Changes in TAFI activity are associated with clinically manifest disorders in hemostatic balance. For example, low activity of the inhibitor is observed in deep venous thrombosis (Martini *et al*., 2006). Studies have found that TAFIa levels determine the risk of cardiovascular mortality in patients with acute coronary disease (Tregouet *et al*., 2009; Morange *et al*., 2005). High TAFI levels were measured in stable angina pectoris and angiographycally confirmed coronary artery disease (Gorog, 2010).

Our study established low plasma TAFI activity in the first twenty-four hours of the clinical presentation of PAF (p <0.001; Figure 1). We found no similar studies in literature to compare with our results. The “pureness” of the groups in terms of diseases and affecting hemostasis drugs give us reason to believe that the results are closely related to the studied disease and not a consequence of other factors.

As already mentioned the TAFI pathway defines the specific molecular link between the inherently opposing processes of coagulation and fibrinolysis. Therefore the study of its activity throws a significant amount of light on the intimate regulatory mechanisms of hemostasis. Low TAFI activity determines a significantly reduced inhibitory effect on fibrinolysis. In this

sense our results suggest an increased activity of the fibrinolytic system in the first twenty-four hours of the clinical presentation of PAF as a result of the reduced inhibition of TAFI. It is well known that the rate of thromboembolic events in these early hours of the disease is negligible (Seet *et al*., 2011). Logically this could be explained by the changes we identified, namely a lower risk of thromboembolic events as a result of reduced inhibition of fibrinolysis. However, it is appropriate to note that in our study TAFI activity was tested only once. This does not give an opportunity to explore the dynamics in the activity of fibrinolysis, which could explain the significant increase in embologenic risk during the later hours of PAF.

In recent years AF is increasingly defined as an inflammation disease. There are inflammatory changes established in the myocardium which are directly related to the structural remodeling of the atria (Frustaci *et al*., 1997). Elevated levels of a number of inflammatory biomarkers such as IL-6, TNF-α, CRP, and certain components of the complement system such as C3 and C4 were measured in plasma (Aviles *et al*., 2003; Celebi et a., 2011; Sata *et al*., 2004; Dernellis *et al*., 2001). The low TAFI activity in our study determines the reduced inactivation of C3a and C5a components of the complement, which in turn leads to enhanced inflammation process.

Conclusion: TAFI activity is significantly reduced in the first twenty-four hours of the clinical presentation of PAF. These early changes suggest their close relationship with the disease. At the same time they are a prerequisite for an increased activity of the fibrinolytic system in plasma, which may explain the low embologenic potential during the first hours of PAF.

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