International Journal of Cardiology 258 (2018) 138–143

Contents lists available at ScienceDirect

International Journal of Cardiology

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

Phosphatidylserine-exposing blood cells and microparticles induce procoagulant activity in non-valvular atrial fibrillation

Lixiu Wang [a,1], Yayan Bi [a,*,1], Muxin Yu [b], Tao Li [b], Dongxia Tong [b], Xiaoyan Yang [b], Cong Zhang [b], Li Guo [b], Chunxu Wang [b], Yan Kou [a], Zengxiang Dong [a], Valerie A. Novakovic [c], Ye Tian [a], Junjie Kou [d,**], Masood A. Shammas [c], Jialan Shi [b,e,***,2]

[a] Department of Cardiology, The First Hospital, Harbin Medical University, Harbin, China
[b] Department of Hematology, The First Hospital, Harbin Medical University, Harbin, China
[c] Department of Research, VA Boston Healthcare System, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
[d] Department of Cardiology, The Second Hospital, Harbin Medical University, Harbin, China
[e] Department of Surgery, VA Boston Healthcare System, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

ARTICLE INFO

Article history:
Received 19 September 2017
Received in revised form 21 January 2018
Accepted 26 January 2018

Keywords:
Non valvular atrial fibrillation
Phosphatidylserine
Blood cell
Microparticle
Procoagulant activity

ABSTRACT

Background: The definitive role of phosphatidylserine (PS) in the prothrombotic state of non-valvular atrial fibrillation (NVAF) remains unclear. Our objectives were to study the PS exposure on blood cells and microparticles (MPs) in NVAF, and evaluate their procoagulant activity (PCA).
Methods: NVAF patients without ($n = 60$) and with left atrial thrombi ($n = 18$) and controls ($n = 36$) were included in our study. Exposed PS was analyzed with flow cytometry and confocal microscopy. PCA was evaluated using clotting time, factor Xa (FXa), thrombin and fibrin formation.
Results: PS^+ blood cells and MPs were significantly higher in NVAF patients without and with left atrial thrombi (both $P < 0.01$) than in controls. Patients with left atrial thrombi showed increased PS^+ platelets, neutrophils, erythrocytes and MPs compared with patients without thrombi (all $P < 0.05$). Moreover, in patients with left atrial thrombi, MPs primarily originated from platelets (56.1%) followed by leukocytes (21.9%, including MPs from neutrophils, monocytes and lymphocytes), erythrocytes (12.2%) and endothelial cells (8.9%). Additionally, PS^+ blood cells and MPs contributed to markedly shortened coagulation time and dramatically increased FXa/thrombin/fibrin (all $P < 0.001$) generation in both NVAF groups. Furthermore, blockade of exposed PS on blood cells and MPs with lactadherin inhibited PCA by approximately 80%. Lastly, we found that the amount of PS^+ platelets and MPs was positively correlated with thrombus diameter (all $p < 0.005$).
Conclusions: Our results suggest that exposed PS on blood cells and MPs play a procoagulant role in NVAF patients. Blockade of PS prior to thrombus formation might be a novel therapeutic approach in these patients.

1. Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia, often leading directly to thromboembolism events, which increases the risk of ischemic stroke and transient ischemic attack to four-fold higher than in patients without AF [1–3]. The pathophysiology of thromboembolism in AF is multifactorial. Non-valvular atrial fibrillation (NVAF) is associated with a prothrombotic state characterized by abnormalities of endothelial function and platelet activation. Indeed, increasing evidence implicates Virchow's triad (abnormal blood stasis, endothelial damage, and changes in blood constituents) as the main cause of prothrombotic state [4,5]. Nevertheless, the molecular and cellular events that are associated with this hypercoagulable state have not been well defined.

Abbreviations: PS, phosphatidylserine; NVAF, non-valvular atrial fibrillation; MPs, microparticles; PCA, procoagulant activity; RDW, red cell distribution width; PMPs, platelet-derived MPs; EMPs, endothelial-derived MPs; LMPs, leukocyte-derived MPs; WBC, white blood cell; RBC, red blood cell; MDP, MP-depleted plasma; TF, tissue factor; PT, prothrombin time; APTT, activated partial thromboplastin time; NMPs, neutrophils-derived MPs; MMPs, monocyte-derived MPs; ErMPs, erythrocyte-derived MPs; PLT, platelet.

* Correspondence to: Y. Bi, Department of Cardiology, The First Hospital, Harbin Medical University, 23 Youzheng Street, Nangang District, Harbin 150001, China.
** Correspondence to: J. Kou, Department of Cardiology, The Second Hospital, Harbin Medical University, 246 Xuefu Road, Nangang District, Harbin 150001, China.
*** Correspondence to: J. Shi, Department of Hematology, The First Hospital, Harbin Medical University, 23 Youzheng Street, Nangang District, Harbin 150001, China.
E-mail addresses: biyayan@163.com (Y. Bi), junjiekou@163.com (J. Kou), jialan_shi@hms.harvard.edu (J. Shi).
[1] The authors contributed equally to this project.
[2] Department of Surgery, Brigham and Women's Hospital, VA Boston Healthcare System, Harvard Medical School, Boston, MA 02132, USA.

https://doi.org/10.1016/j.ijcard.2018.01.116
0167-5273/

Our previous studies and others have shown that elevated phosphatidylserine (PS) exposure on microparticles (MPs) and cells plays a critical role in the thrombotic risk associated with some disorders, including nephrotic syndrome and non-ST-elevated myocardial infarction [6,7]. PS, an anionic phospholipid that is normally sequestered on the inner leaflets of cell membranes, is flopped to the outer leaflet of the cell membrane during cell activation or apoptosis [8]. PS provides a catalytic surface to support the assembly of blood coagulation factors, thereby promoting activation of the coagulation cascade and thrombin generation [9]. Previous studies have shown that AF patients have increased expression of P-selectin on platelets, elevated levels of inflammatory markers, and higher red cell distribution width (RDW), indicating the activation of blood cells [10–13]. However, whether exposed PS on blood cells contributes to the procoagulant state in NVAF patients remains to be investigated.

Circulating MPs are small vesicles with diameter 0.1–1 μm that are shed from apoptotic or activated cells, which display parent cell membrane proteins and PS due to loss of membrane asymmetry [8]. Ederhy S et al. have shown that annexin V-positive MPs, platelet-derived MPs (PMPs), endothelial cell-derived MPs (EMPs) and leukocyte-derived MPs (LMPs) are significantly higher in NVAF patients than in healthy controls [11,14–16]. Previous studies showed that the most common cardiac source of embolism is the left atrium and its appendage [17]. However, relatively little is known about the levels of MPs in NVAF patients with left atrial thrombi. Furthermore, it is not entirely clear whether PS exposure on MPs contributes to the hypercoagulable state of NVAF with and without left atrial thrombi, and how MPs affect hemostatic balance in these patients.

In this study, we hypothesized that PS-positive (PS^+) blood cells and MPs were associated with the prothrombotic state of NVAF patients. Lactadherin is a sensitive probe for detecting and blocking exposed PS on MPs and their associated cells [18–20]. In the present study, we used lactadherin to measure the extent of PS exposure on blood cells and characterize the number and cellular origin of PS^+ MPs from NVAF patients with and without left atrial thrombi. Moreover, coagulation time, FXa/thrombin and fibrin formation on these blood cells and MPs were measured. In order to define the relative contribution of PS to the coagulation process, lactadherin was used to block PS at the surface of blood cells and MPs. Our results point to the important role of PS-exposing blood cells and MPs in hypercoagulability in NVAF patients.

2. Methods

2.1. Study population

The newly diagnosed 18 NVAF patients with left atrial thrombi (confirmed by 12-lead ECG and transthoracic echocardiography) and 60 risk factor-matched (RF) NVAF patients without left atrial thrombi and 36 RF-matched controls were consecutively recruited from the First and Second Hospital of Harbin Medical University between July 2015 and March 2017. Exclusion criteria for all patients were: valvular heart diseases, history of stroke, history of coronary artery disease and heart failure, history of thromboembolism, acute and chronic inflammatory diseases, history of surgical procedure within three months of presentation, hepatic or renal disorders, autoimmune or malignant diseases. Considering the effects of anticoagulant and antiplatelet drugs administration on circulating MPs release and their procoagulant activity as demonstrated by previous studies [21], we only enrolled newly diagnosed NVAF patients who never initiated anticoagulant or antiplatelet therapy at the time of blood sampling in the present study.

Risk factors include age, gender, hypertension, diabetes mellitus, dyslipidemia, and a smoking history. The RF controls included patients with no history of AF, no treatment of anticoagulant, and one or more risk factors. Blood samples of all patients and RF-matched controls were included for comparative assessment in the study. After admission, all patients received antiplatelet and anticoagulant treatment after the initial blood draw. The approval from Research Ethics Committees of our hospital was obtained for all participants before the start of the study, in accordance with the Helsinki Declaration; and written informed consent was obtained from all the participants during enrollment.

2.2. Experimental procedures

Blood collection, isolation of blood cells and MPs, flow cytometry and confocal microscopy analysis, coagulation time, FXa, prothrombinase, fibrin formation and inhibition assays are described in detail in the Suppl. Material.

2.3. Statistical analysis

Numerical variables were tested for normal distribution with the Kolmogorov-Smirnov test. Data are expressed as mean ± SD and statistically analyzed using a Student *t*-test or ANOVA as appropriate. Correlations between two continuous variables were performed using Pearson correlation coefficients, and Spearman rank correlations were used to detect discrete variables. Categorical variables were compared using the $\chi 2$ test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Participants characteristics

Clinical characteristics of patients and controls are presented in Table 1. Patients had significantly shorter prothrombin time (PT), activated partial thromboplastin time (APTT), and higher levels of D-dimer when compared with controls, indicating a hypercoagulable state in NVAF patients. For echocardiographic variables, we found that NVAF patients had significantly larger left ventricular end-diastolic diameter and left ventricular end-systolic diameter compared to controls. There were no significant differences between NVAF patients with and without thrombi. Additionally, the left atrial diameter of NVAF patients with thrombi was significantly higher than that in patients without

Table 1
Characteristics of controls, NVAF without and with left atrial thrombi at inclusion.

	Controls (n = 36)	NVAF (n = 60)	NVAF with Thr (n = 18)
Age (year)	59.26 ± 10.32	60.12 ± 10.82	61.37 ± 11.26
Gender, male, n (%)	18 (50.0)	32 (53.3)	10 (55.6)
Body mass index (kg/m^2)	25.9 ± 5.7	26.3 ± 5.1	25.7 ± 5.3
Smoking, n (%)	21 (58.3)	34 (56.7)	10 (55.6)
Dyslipidemia, n (%)	6 (16.7)	11 (18.3)	4 (22.2)
Hypertension, n (%)	15 (41.7)	24 (40.0)	8 (44.4)
Diabetes mellitus, n (%)	11 (30.6)	19 (31.7)	6 (33.3)
Platelet counts (10^9/L)	193.54 ± 88.71	239.31 ± 60.16	232.93 ± 67.59
Erythrocyte counts (10^{12}/L)	4.11 ± 0.88	4.31 ± 0.34	4.52 ± 0.43
Leukocyte counts (10^9/L)	6.84 ± 1.62	8.90 ± 2.36	10.79 ± 3.34
Neutrophil (%)	62.75 ± 4.81	75.12 ± 4.34[#]	75.31 ± 4.18[*]
Monocyte (%)	5.37 ± 1.53	5.98 ± 1.39	5.61 ± 1.53
Lymphocyte (%)	29.91 ± 4.38	20.46 ± 3.02	19.09 ± 3.76
Prothrombin time (s)	12.10 ± 1.13	9.38 ± 1.41[#]	9.82 ± 1.91[*]
APTT (s)	27.50 ± 3.42	20.49 ± 3.06[#]	18.47 ± 5.15[*]
D-dimer (mg/l)	0.43 ± 0.19	2.76 ± 0.47[#]	3.80 ± 0.69[*]
Fibrinogen (g/l)	2.81 ± 0.75	3.59 ± 0.69	4.76 ± 0.83[*]
Echocardiographic variables	–	–	–
LVEDD (cm)	4.31 ± 0.49	5.53 ± 0.48[#]	5.83 ± 0.37[*]
LVESD (cm)	3.95 ± 0.53	4.56 ± 0.43[#]	4.47 ± 0.51[*]
LAD (cm)	3.35 ± 0.49	3.74 ± 0.38	4.85 ± 1.78[*]
RAD (cm)	2.83 ± 0.09	3.14 ± 0.51	3.29 ± 0.44
LVEF (%)	63.0 ± 4.9	57.2 ± 7.1	54.9 ± 9.3
Thrombus size (cm)	–	–	5.94 ± 1.81[*]
CHA_2DS_2-VASc, n (%)	–	–	–
Low (score 0)	–	21 (35.0)	7 (38.9)
Intermediate (score 1)	–	14 (23.3)	4 (22.2)
High (score 2–9)	–	25 (41.7)	7 (38.9)
Pharmacological therapy	–	–	–
ACE inhibitor or ARB, n (%)	5 (13.9)	20 (33.3)[#]	8 (44.4)[*]
β-blocker, n (%)	7 (19.4)	19 (31.7)[#]	7 (38.9)[*]
Statin, n (%)	6 (16.7)	13 (61.7)[#]	5 (27.8)
Ca antagonists, n (%)	4 (11.1)	18 (30)[#]	10 (55.6)[*]
AF subtype, n (%)	–	–	–
Paroxysmal AF	–	19 (31.7)	6 (33.3)
Persistent AF	–	41 (68.3)	12 (66.7)

Data are presented as mean ± SD or number (%). NVAF: non-valvular atrial fibrillation; Thr: thrombi; APTT: activated partial thromboplastin time; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; LAD: left atrial diameter; RAD: right atrial diameter; LVEF: left ventricular ejection fraction; CHA_2DS_2-VASc, congestive heart failure, hypertension, age ≥ 75 years, diabetes, previous stroke or systemic thromboembolism or transitory ischemic attack, vascular disease, age 65–74 years, sex category.

[#] $P < 0.001$, NVAF vs. Controls.

[*] $P < 0.001$, NVAF with Thr. vs. Controls.