Platelets

ISSN: 0953-7104 (Print) 1369-1635 (Online) Journal homepage: https://www.tandfonline.com/loi/iplt20

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To cite this article: Jonas S.S.G. De Jong, Rienk Nieuwland, Joost C.M. Meijers, Suzanne J. Korporaal, Jan Willem N. Akkerman, Arthur A.A.M. Wilde & Lukas R.C. Dekker (2014) Altered platelet contents in survivors of early ischemic ventricular fibrillation: Preliminary findings, Platelets, 25:1, 71-74, DOI: 10.3109/09537104.2012.754418

To link to this article: https://doi.org/10.3109/09537104.2012.754418

Published online: 09 Jan 2013.

Platelets,; February 2014; 25(1): 71–74

ISSN 0953-7104 print/ISSN 1369-1635 online
DOI: 10.3109/09537104.2012.754418

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SHORT COMMUNICATION

Altered platelet contents in survivors of early ischemic ventricular fibrillation: Preliminary findings

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Abstract

Early ischemic ventricular fibrillation (VF) in the setting of an acute myocardial infarction (AMI) due to thrombotic coronary occlusion remains a major health problem. Several animal studies have shown that platelet-dense granule contents released during thrombus formation can induce arrhythmias. We hypothesize that the platelet release reaction is involved in the predisposition to early ischemic VF. A case-control study was performed in patients who survived VF during a first AMI (''cases,'' $n = 26$) and in patients with one previous AMI without arrhythmias (''controls,'' $n = 24$). All patients were on aspirin 100 mg OD. Baseline platelet activation was assessed with flow cytometry. Response to activation was assessed with aggregometry, flow cytometry and PFA-100 analysis. Differences in platelet contents and content release were assessed by labeling platelet-dense granules with mepacrine and by measuring serotonin and ADP/ATP content. Patient and infarct characteristics and baseline platelet function tests were similar between groups. The mean time from event was 4.9 (±3.2) years among cases and 4.7 (±2.7) years among controls. Dense granule release was similar in cases versus controls. Platelet serotonin content in cases was higher than in controls (611 ± 118 ng/10E^9 platelets *vs.* 536 ± 141 ng/10^9, $p = 0.048$). Even years after the event, elevations in the platelet dense granule contents between VF survivors and controls may be detected. These preliminary findings shed new light on the pathophysiological mechanisms underlying ischemic VF, as platelet-dense granules may contain mediators of early ischemic VF risk.

Keywords: *Platelets*, *myocardial infarction*, *heart arrest*, *ventricular fibrillation*

Introduction

Sudden cardiac death (SCD) remains one of the most prevalent modes of death in the industrialized countries [1], claiming almost a million deaths annually in Western Europe and the United States combined. Ventricular fibrillation (VF) in the setting of coronary artery disease is the most common underlying arrhythmia. In a large proportion of cases, SCD is caused by early ischemic VF, i.e., VF in the setting of a first acute myocardial infarction (AMI) and in the absence of heart failure [1]. A potential modifier of the risk of VF could be related to platelet activation. Animal experiments have shown that platelet activation increases the susceptibility of the ischemic myocardium to VF [2, 3]. Many of the substances released by platelets contained in dense granules, α-granules and lysosomal granules can alter the electrophysiological properties of the heart in various animal species [4, 5].

In the present study, we aimed to explore whether the platelet release reaction in patients with VF prior to reperfusion for a first STEMI differs from that in patients with an arrhythmia-free STEMI. We hypothesized that the platelet-release reaction in VF patients could be affected by different factors: altered platelet activation at rest, altered response of platelets to activation and differences in products released by activate platelets. We performed a series of tests to assess these factors.

Materials and methods

All patients were included in the AGNES (Arrhythmia Genetics in the NEtherlandS) case-control study [6], and signed informed consent in accordance with the principles outlined in the Helsinki Declaration of 1975, as revised in 2008. The study was approved by the institutional Medical Ethics Committee (reference number #06/002). All patients were on chronic anti-platelet therapy (aspirin 100 mg OD). All blood draws were performed by a single investigator between 10 and 11 am with a 19 G needle without vacuum in a single blood draw to prevent circadian platelet function influence and platelet activation.

Standard hematology measurements were performed on a Beckman Coulter Ac·T diff2 Hematology Analyzer. Prothrombin time, activated partial thromboplastin time and fibrinogen were measured on an STA-Evolution analyzer. Protein C activity, antithrombin, fibrinogen and Von

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(Received 5 August 2012; revised 25 November 2012; accepted 27 November 2012)

Table I. Baseline platelet activation, response to activation and platelet contents.

	Cases ($n = 26$)	Controls ($n = 24$)	p-value[a]
Platelet aggregation			
Slope 1 μmol/L ADP (%/min)	35 ± 25	28 ± 18	0.243
Slope 5 μmol/L ADP (%/min)	77 ± 15	79 ± 13	0.775
Slope 5 μmol/L TRAP (%/min)	84 ± 14	89 ± 13	0.233
PFA-100 measurements			
PFA-ADP (s)	94 ± 45	106 ± 64	0.777
PFA-EPI (s)	180 ± 83	204 ± 95	0.752
VASP PRI (%)	78 ± 4	77 ± 4	0.514
Flow cytometry			
P-selectin-exposing platelets, baseline[b]	3.98 ± 1.41	3.60 ± 1.48	0.349
P-selectin-exposing platelets, TRAP[b]	86 ± 11	90 ± 7.4	0.143
Monocyte count	374 ± 92	357 ± 77	0.491
CD61 + monocytes (% of monocytes)	7.7 ± 4.4	6.1 ± 3.7	0.450
Platelet mepacrine fluorescence[b]	13.0 ± 2.6	14.5 ± 3.9	0.124
Mepacrine release[c]	0.42 ± 0.12	0.45 ± 0.11	0.376
Platelet contents			
ADP[d]	3.1 ± 0.8	3.1 ± 0.7	0.866
ATP[d]	4.3 ± 0.7	4.5 ± 1.0	0.362
ADP/ATP ratio	1.4 ± 0.2	1.5 ± 0.2	0.547
Serotonin (ng/10^9)	611 ± 118	536 ± 141	0.048*

Notes: Values are mean ± SD.
[a]cases *vs.* controls.
[b]arbitrary units.
[c]calculated as the fraction of mepacrine fluorescence remaining after platelet activation with TRAP.
[d]the unit is μmol/10^{11} thrombocytes.
TRAP = thrombin receptor activating peptide; PFA-ADP/PFA-EPI = PFA·100 using ADP/epinephrine; VASP PRI = VASP platelet reactivity index; ADP: adenosine diphosphate; ATP: adenosine triphosphate.
*$p < 0.05$: statistically significant.

Willebrand Factor activity were measured on an ACL Top analyzer. ELISA tests were used for thrombin-antithrombin complexes and d-dimer measurements. Light-transmittance aggregometry was used to assess response of platelets to different activating substances (Table I). Baseline P-selectin expression and P-selectin expression after platelet activation with TRAP was measured with flow cytometry using a Calibur flow-cytometer (B&D, San José, CA, USA). Beta-thromboglobulin (primarily stored in alpha granules) and platelet factor 4 were measured in plasma as markers of baseline platelet activity. Vasodilator-stimulated phosphoprotein (VASP) phosphorylation was measured by flow cytometry as described before and expressed as VASP PRI (platelet reactivity index; Table I) [7]. The amount of VASP phosphorylation reflects the P2Y12 receptor status. Platelets were incubated with both VASP-P-FITC and CD61-APC. Analysis of cell counts was performed using the software program CELLquest.

Response of platelets to activation by ADP and epinephrine was assessed with a PFA-100 (Dade Behring, Germany). Dense granule contents and release was assessed by labeling platelet-dense granules with mepacrine [8]. Flow cytometry was used to assess the amount of mepacrine fluorescence. Platelet-rich plasma was labeled with CD61-APC, CD62p-PE and mepacrine. Release of dense granules was assessed by measuring the difference in mepacrine-labeled platelet fluorescence before and after activation with TRAP. We could thereby assess the number of remaining dense granules in platelets after they were activated. Serotonin was used as a marker of dense-granule release. Platelets were pelleted and sonified in PBS, and thereafter all protein was precipitated with HClO4. Serotonin concentration was measured by fluorimetry. The amount of serotonin per platelet was normalized by dividing the total amount of serotonin by the number of platelets. Platelet ATP and ADP contents were assessed by measuring ATP and ADP with the luciferin-luciferase technique in a Packard Pico-lit luminometer.

The data are presented as frequency (number and percentage), mean ± standard deviation (SD) or median (inter-quartile range). Normal distribution of data was tested with the Kolmogorov–Smirnov Z test. Normal distributed data were analyzed with a Student's t-test. Non-normal distributed data were analyzed with a Mann–Whitney U test. Statistical significance was defined as $p < 0.05$. Analyses were performed using SPSS version 17.0 (SPSS Inc, Chicago, IL).

Results

Baseline and infarct characteristics

Cases ($n = 26$) and controls ($n = 24$) were well matched. Average age was 61 ± 11 vs. 64 ± 7 years; 89% and 75% were men, respectively. All patients were on aspirin, and most on beta blockers (65% *vs.* 86%) and statins (56% *vs.* 59%). The STEMI occurred at a mean of 4.8 years earlier. The characteristics of MI in both groups were comparable. One control patients was using a selective serotonine re-uptake inhibitor.

Results of platelet tests

There were no statistically significant differences between the two groups in any laboratory or coagulation test (data not

shown). Platelet activity as measured by light transmittance aggregometry, PFA-100 and VASP phosphorylation did not differ between cases and controls (Table I). Mepacrine fluorescence in unstimulated platelets and stimulated platelets did not differ between cases and controls. Furthermore, the number of CD62p positive platelets and the number of monocyte-platelet complexes were similar in both groups. Dense granule release as assessed by the difference in mepacrine-labeled platelet fluorescence before and after activation with TRAP was similar in cases vs. controls. Platelet ADP and ATP content and ADP/ATP fraction did not differ between cases and controls. Platelet serotonin was significantly higher in cases *versus* controls (611 ± 118 ng/10^9 *vs.* 536 ± 141 ng/10^9; $p = 0.048$; Table I). Exclusion of the control patient who was on selective serotonin reuptake inhibitors (SSRIs, anti-depressant drugs) did not influence this statistic.

Discussion

These results provide preliminary evidence to suggest that even five years after STEMI, and apparently unaffected by aspirin use, platelet serotonin may be higher among patients experiencing VF during a first STEMI compared with those who have previously experienced an STEMI without arrhythmia. We therefore suggest that through direct and indirect (induction of coronary vasospasm) effects, serotonin could worsen the already-present ischemic substrate for arrhythmias. Serotonin transport in blood is almost exclusively by platelets. During platelet activation this serotonin is readily released. Animal studies in an MI model have shown that serotonin concentration in the affected coronary artery and its direct surroundings increases 18–27 fold. In the same experiments, serotonin-induced cyclic coronary flow variations by coronary vasoconstriction [9]. Serotonin has direct inotropic, lusitropic, and pro-arrhythmic effects on human ventricular cardiomyocytes that contain serotonin receptors [10, 11] and it is increased in patients with coronary artery disease [12]. Treatment of post myocardial infarction depression with SSRIs reduces all cause mortality [13]. Serotonin levels can be extremely elevated in patients with serotonin producing carcinoid tumors or SSRI overdosing. Arrhythmias are not a common finding in carcinoid, probably because inactivation of humeral substances by the lung protects the left heart side. Concurringly right sided valve disease is a much more common hallmark of carcinoid [14]. In SSRI overdosing QTc prolongation is often observed, which is pro-arrhythmic [15]. Serotonin could also be seen as a marker of dense granule release. It is possible that it is not serotonin but one of the other dense-granule contents (e.g., histamine, pyrophosphate) that mediates arrhythmias during MI. Cases could release all their dense granule contents more readily during platelet activation. However, our experiments with mepacrine-labeled dense granules could not detect evidence for this.

This study is unique in the fact that we thoroughly assessed the different mechanisms involved in the platelet activation reaction in cases and controls. The most important limitations of this study is the small number of patients involved, which was an inevitable consequence of the elaborate and stringent requirements of the protocol necessary to perform this study. All patients were taking aspirin and their current platelet activation reaction might not fully reflect the platelet status at the time of the initial event. Quantifying dense granules using an electron microscope would have been a valuable addition. Differences in the effects of platelet products could also result from differences in response from target cells (coronary endothelium and cardiomyocytes, for example), which is beyond the scope of this article.

Years after the event and irrespective of anti-platelet therapy, these results provide preliminary evidence to suggest that a difference in serotonin content may be detected between patients with a first AMI who also experienced a VF and first AMI patients without VF. It is hypothesized that serotonin may induce arrhythmias directly or by inducing coronary spasm, or it may be a marker of dense-granule release that contains other pro-arrhythmic substances. After confirmation of these findings in a larger study, further research is necessary to determine which factors released by platelets have the strongest arrhythmic effect. Our preliminary findings shed new light on the pathophysiological mechanisms that may be involved in the occurrence of VF during MI.

Acknowledgments

We thank M. Tanck for statistical advice, R. Danks for proofreading, and M.C.L. Schaap and C.M. Hau for technical assistance.

Declaration of interest: Financial support for this work came from the Netherlands Heart Foundation (2001-D019) and the Leducq Foundation (Alliance against SCD, CV05), for which we want to express our grateful acknowledgement. Suzanne Korporaal is financed by the Landsteiner Foundation for Blood Transfusion Research, project nr. 0912F.

The authors report no conflicts of interest

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