Platelets

ISSN: 0953-7104 (Print) 1369-1635 (Online) Journal homepage: informahealthcare.com/journals/iplt20

The membrane expression of P-selectin, but not monocyte-platelet aggregates, is influenced by variability in response to aspirin in patients with coronary artery disease

N. Kuzniatsova, E. Shantsila, G.Y.H. Lip & A.D. Blann

To cite this article: N. Kuzniatsova, E. Shantsila, G.Y.H. Lip & A.D. Blann (2014) The membrane expression of P-selectin, but not monocyte-platelet aggregates, is influenced by variability in response to aspirin in patients with coronary artery disease, Platelets, 25:2, 142-143, DOI: 10.3109/09537104.2012.739720

To link to this article: https://doi.org/10.3109/09537104.2012.739720

Published online: 05 Dec 2012.

Platelets, March 2014; 25(2): 142–143

ISSN 0953-7104 print/ISSN 1369-1635 online
DOI: 10.3109/09537104.2012.739720

informa
healthcare

LETTER TO THE EDITOR

The membrane expression of P-selectin, but not monocyte-platelet aggregates, is influenced by variability in response to aspirin in patients with coronary artery disease

N. Kuzniatsova, E. Shantsila, G.Y.H. Lip & A.D. Blann

Department of Medicine, University of Birmingham Centre for Cardiovascular Sciences, City Hospital, Birmingham, B18 7QH, UK

Keywords: Aspirin resistance, coronary artery disease, flow cytometry, platelet aggregation, P-selectin, monocyte-platelet aggregates

Although anti-platelet therapy is the mainstay of cardiovascular disease, the fact that thrombosis still occurs despite the use of 75 mg/d aspirin implies that in some subjects (perhaps 10–20%) this agent is sub-effective. This variability in response to aspirin has led to the concept of a 'suboptimal response to aspirin', also described as 'high on-treatment platelet reactivity' or 'aspirin resistance' [1]. This suboptimal response in patients undergoing percutaneous coronary intervention has been associated with increased risk of thrombosis and increased major adverse clinical events in long-term follow-up [2]. However, research into aspirin variability (and so the concept of resistance) is confounded by variation in the daily dose of aspirin taken by patients, and the lack of a clear consensus of a definition, the latter partly due to numerous laboratory methods that include aggregometry (the most common method)[3].

Quantification of monocyte-platelet aggregates (MPAs) is an additional method that may prove useful in thrombosis research [4]. Increased MPAs are present in a variety of cardiovascular conditions, and although several workers compared MPAs with soluble and membrane expression of P-selectin [5], none have adequately addressed their possible link with aspirin resistance, pertinent as monocytes are sensitive to this drug [6]. We therefore hypothesised that variability in the response to aspirin would influence soluble and membrane expression of P-selectin, and MPAs. We tested this hypothesis in patients with existing coronary artery disease (CAD), and therefore on 75 mg aspirin daily.

We recruited 50 patients (12 women, 11 diabetics) with proven stable CAD. Twenty-seven patients had a history of angina, 15 a previous myocardial infarction, 21 had coronary artery bypass grafting, 11 a stroke/transient ischaemic attack, 8 peripheral artery disease and 18 had percutaneous coronary intervention. No patient was within three months of their index event. Drugs prescribed to the CAD patients were a statin to 45 (so that total cholesterol was mean 4.2 standard deviation (SD) 0.9 mmol/l), an angiotensin converting enzyme inhibitor or angiotensin receptor blocker to 40, a beta blocker to 30, a calcium channel blocker to 14, a diuretic to 15, a nitrate to 11 (giving SBP/DBP 138 [23] and 74 [12] mmHg) and insulin to 2 (giving HbA1c 6.3 [0.6]%). Exclusion criteria were current use of oral or parenteral anticoagulation or other anti-platelet drugs, bleeding abnormalities and/or significant hepatic, neoplastic, renal, connective tissue disease or inflammatory disease. The project had the approval of the Local Research Ethics Committee and written informed consent was obtained from each subject.

Venous blood was taken into 3.2% sodium citrate for light transmission aggregometry (LTA) on 270 µl aliquots of platelet rich plasma (1000 rpm, 10 minutes) with a 4-channel PAP aggregometer (Alpha Labs, Basingstoke, UK) according to standard protocols using 30 mL of arachidonic acid (Sigma Aldrich, UK, 0.5 mg/ml). Percentage of light transmission was collected at three minutes after the addition of the agonist. Thromboxane B_2 was assessed in serum, and soluble P-selectin was measured in citrated plasma by ELISA (R&D Systems, Abingdon, UK).

MPAs and platelet P-selectin expression were measured by flow cytometry (FACScalibor, Becton Dickinson, Oxford, UK). Briefly, the platelet cloud was identified in forward and side scatter, gated and platelet identity confirmed by CD42a-PerCP (all mAbs from Becton Dickinson, Oxford, UK). To quantify activated platelets, P-selectin was expressed as the percentage of platelets expressing the molecule above isotype control, and by the mean fluorescence intensity (MFI) at rest and after stimulation with arachidonic acid at 125 µg/ml for five minutes. MPAs were defined in a separate forward and side scatter gate by events co-staining with CD42a-PerCP and with CD14-PE [7]. Data distributed normally are presented as mean and SD and analysed by student's *t*-test. Data distributed

Correspondence: A.D. Blann, Department of Medicine, University of Birmingham Centre for Cardiovascular Sciences, City Hospital, Birmingham, B18 7QH, UK. Tel: 00 44 121 507 5076. E-mail: a.blann@bham.ac.uk

(Received 9 October 2012; accepted 10 October 2012)

Table I. Effect of aspirin resistance or sensitivity.

	Aspirin resistance ($n=23$)	Aspirin sensitivity ($n=27$)	p-value
LTA response to arachidonic acid (%)	26.0 (23.0–28.0)	14.0 (10.0–16.5)	<0.001
Thromboxane (pg/ml)	3.4 (2.3–3.9)	3.5 (2.4–4.9)	0.455
Soluble P-selectin (ng/ml)	80.8 (34.1)	68.9 (20.8)	0.137
Monocyte count (cells/$\times 10^6$/l)	541 (122)	603 (196)	0.184
Monocyte/platelet aggregates (cells/$\times 10^6$/1)	113 (87)	113 (52)	0.993
Resting P-selectin expression (%)	28.1 (11.0)	21.9 (6.1)	0.016
Resting P-selectin expression (MFI, arbitrary units)	37.7 (11.2)	31.8 (3.1)	0.012
Stimulated P-selectin expression (%)	46.6 (16.8)	37.8 (13.7)	0.063
Stimulated P-selectin expression (MFI, arbitrary units)	70.2 (34.4)	53.4 (24.8)	0.066

Notes: LTA = light transmission aggregometry, MFI = mean fluorescence intensity. Data presented as mean (SD) (analysed by *t*-test) or median (interquartile range) (analysed by the Mann–Whitney *U*-test).

non-normally are presented as median and inter-quartile range and analysed by the Mann–Whitney *U*-test. Data were correlated with Spearman's method. $P<0.05$ was considered statistically significant.

Table I shows analysis where the aspirin resistance or sensitivity is dichotomised as an LTA response to arachidonic acid of >/< 20% [3], under which conditions, 23 patients (46%) were aspirin resistant. There was no significant difference in soluble P-selectin, monocyte count or MPAs in the aspirin resistant patients compared to those sensitive to aspirin. Resting platelet P-selectin expression was significantly lower in the patients whose response to aspirin was normal, but this difference was not significant in platelets stimulated by arachidonic acid. It has been argued that some resistance may be poor compliance, but no difference in thromboxane levels between the resistant group and the sensitive group implies good compliance. Overall, number of MPAs correlated significantly with soluble P-selectin ($r=0.38$, $p=0.006$) but not with any other platelet index.

Interest in MPAs as a marker of cardiovascular disease is growing although their pathophysiological significance is unknown [8]. They may represent the scavenging of effete or activated platelets by phagocytic monocytes, or perhaps a signalling mechanism to change the monocyte phenotype. The correlation between MPAs and soluble P-selectin may reflect the former since soluble P-selectin is a marker of platelet activation. Notably, MPA formation *in vitro* can be inhibited by antibodies to P-selectin and its ligand [8]. In another setting, MPAs may be used to dissect pathophysiology and differing effects of alternative methods of suppressing platelet function in those at risk of adverse events [9]. But whatever the function of MPAs is, we suggest that variability in an individual's response to aspirin does not markedly influence this marker.

Despite small numbers, we found that aspirin sensitivity defined by a dichotomous 20% LTA response was associated with lower expression of membrane P-selectin by resting, but not arachidonic acid stimulated cells. This implies that at least some component of the mobilisation of the alpha granule is linked to the cyclo-oxygenase pathway, which is likely as aspirin clearly does not fully suppress this pathway, and supports other data showing continuing alpha degranulation despite the use of this drug [10].

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Kuzniatsova N, Shantsila E, Blann A, Lip GY. A contemporary viewpoint on 'aspirin resistance'. Ann Med 2012. PMID: 22380694. E-publication prior to print.
2. Breet NJ, van Werkum JW, Bouman HJ, Kelder JC, et al. High on-aspirin platelet reactivity as measured with aggregation-based, cyclooxygenase-1 inhibition sensitive platelet function tests is associated with the occurrence of atherothrombotic events. J Thromb Haemost 2010;8:2140–2148.
3. Lordkipanidzé M, Pharand C, Schampaert E, Turgeon J, Palisaitis DA, Diodati JG. A comparison of six major platelet function tests to determine the prevalence of aspirin resistance in patients with stable coronary artery disease. Eur Heart J 2007;28:1702–1708.
4. Shantsila E, Lip GY. The role of monocytes in thrombotic disorders. Insights from tissue factor, monocyte-platelet aggregates and novel mechanisms. Thromb Haemost 2009;102:916–924.
5. Michelson AD, Barnard MR, Krueger LA, Valeri CR, Furman MI. Circulating monocyte-platelet aggregates are a more sensitive marker of in vivo platelet activation than platelet surface P-selectin: Studies in baboons, human coronary intervention, and human acute myocardial infarction. Circulation 2001;104:1533–537.
6. Crutchley DJ. Effects of inhibitors of arachidonic acid metabolism on thromboplastin activity in human monocytes. Biochem Biophys Res Commun 1984;119:179–184.
7. Tapp LD, Shantsila E, Wrigley BJ, Pamukcu B, Lip GY. The CD14++CD16+ monocyte subset and monocyte-platelet interactions in patients with ST-elevation myocardial infarction. J Thromb Haemost 2012;10:1231–1241.
8. Sarma J, Laan CA, Alam S, Jha A, Fox KA, Dransfield I. Increased platelet binding to circulating monocytes in acute coronary syndromes. Circulation 2002;105:2166–2171.
9. Braun OO, Johnell M, Varenhorst C, James S, Brandt JT, Jakubowski JA, Winters KJ, Wallentin L, Erlinge D, Siegbahn A. Greater reduction of platelet activation markers and platelet-monocyte aggregates by prasugrel compared to clopidogrel in stable coronary artery disease. Thromb Haemost 2008;100:626–633.
10. Pernerstorfer T, Stohlawetz P, Stummvoll G, Kapiotis S, Szekeres T, Eichler HG, Jilma B. Low-dose aspirin does not lower in vivo platelet activation in healthy smokers. Br J Haematol 1998;102:1229–1231.