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REGULAR ARTICLE

Platelet P-selectin and platelet mass, volume and component in sickle cell disease: Relationship to genotype

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Abstract

Background and purpose: Excess platelet activation (e.g. increased soluble P selectin [sPsel] and beta thromboglobulin [β-TG]) is well established in sickle cell disease (SCD) and may contribute to the prothrombotic/hypercoagulable state and vascular occlusion characteristic of the disease. We hypothesised altered whole platelet P-selectin (pPsel), and morphological platelet indices mass, volume and component in SCD and two of its major genotypes.
Methods: We recruited 35 SCD patients [mean age 31 years, 54% men]. Of these, 16 had homozygous sickle cell (HbSS) disease and 19 had sickle-haemoglobin-C (HbSC) disease. Patients were compared with 29 subjects with normal haemoglobin (HbAA) matched for age and ethnicity. Platelet mass, volume and component were measured by flow cytometry, pPsel in platelet lysate, sP-sel and β-TG by ELISA.
Results: SCD patients had lower pP-sel and mean platelet volume (MPV) but elevated platelet component (MPC), and, as expected, elevated platelet count, and sP-sel (all $p<0.05$) compared to HbAA subjects. In both groups, pPsel correlated with MPV, and MPV correlated positively with mean platelet mass (MPM) and negatively with MPC. sPsel correlated with platelet count only in SCD, not in the controls. Platelet count alone was different (higher) in HbSS compared to HbSC, and sPsel correlated with platelet count only in HbSC disease, not in HbSS disease.
Conclusion: Patients with SCD have various abnormalities in their platelets regardless of genotype: there are more numerous platelets, which are smaller, contain less P selectin per cell, but have a higher concentration of granules than

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those of HbAA subjects. These differences may mark and/or promote the prothrombotic state in SCD.
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Introduction

Microvascular occlusion is the pathophysiological hallmark of sickle cell disease (SCD) and is a complex multifactoral process [1]. One of the major factors contributing to vascular occlusion is thought to be a hypercoagulable or prothrombotic state [2–4]. This may be attributable to factors such as abnormalities in the coagulation pathway leading to increased thrombin generation, depletion of the anticoagulant proteins C and S [5,6], and/or to other changes such as increased platelet count and activity [6–12].

More recently, increased expression of surface P-selectin as assessed by flow cytometry [9,10,13] or the plasma level of soluble P-selectin [11,12,14,15] are becoming recognised as evidence of platelet activation (n.b. activation in the pro-thrombotic, as opposed to metabolic/intracytoplasmic, meaning). P-selectin (CD62P), an adhesion molecule facilitating the adhesion of platelets to leucocytes and to the endothelium, is a component of the membrane of the endothelial Weibel–Palade body and the membrane of the platelet alpha and dense granules. During activation and adhesion, platelets mobilise their alpha granules so that P-selectin appears at the surface, and may subsequently be shed into the plasma. This shed P-selectin is measured as *soluble* P-selectin (sP-selectin), and it is now believed that most, if not all, of this sP-selectin in the plasma is of platelet origin and, to some extent, parallels levels of plasma β-thromboglobulin, a more established platelet marker [10]. We have recently reported a novel method of quantifying the total amount of P-selectin in platelets (pP-selectin) by lysing a set number of cells with the detergent Triton X-100, and measuring P-selectin by ELISA in the resultant lysate, although the value of this method is as yet unclear [16].

Recent advances in moving conventional flow cytometry (e.g. FACScan) into 'routine' haematology autoanalysers have enabled the measurement of additional indices of platelet biology [17,18]. Using two-angle forward and side scatter, these permit assessment of aspects of platelet morphology such as mean platelet volume (MPV) and component/density (MPC). The latter, possibly reflective of cell activation [19,20], consists of solids within the platelet, such as proteins, lipids and carbohydrates [18]. The mass of each platelet (i.e. MPM) is calculated by multiplying MPV and MPC. These new types of data are lacking in SCD, whatever the genotype, and may provide additional insight into the pathophysiological role of platelets in this condition.

We hypothesised (a) that, like sPselectin [11,12], the amount of P-selectin per platelet (pP-selectin) would be elevated in SCD compared to healthy controls, and that this increase would parallel the severity of SCD, and (b) that other platelet indices (mass, volume and component) would differ between the subject groups. We used another platelet activation marker, beta thromboglobulin, as a comparator [10]. To test these hypotheses, we examined platelets from patients with SCD (patients with homozygous sickle cell (HbSS) and the clinically milder sickle-haemoglobin C (HbSC) disease) and controls with normal haemoglobin (AA) genotype, of comparable age and matched for ethnic origin with the SCD patients.

Materials and methods

Patients with SCD who attended the Sickle Cell and Thalassemia Centre at City Hospital, Birmingham, were approached to participate in this study. Diagnosis (HbSS or HbSC) was proven by routine high performance liquid chromatography. The control subjects with normal haemoglobin (AA) genotype, matched for ethnic origin with the SCD patients and of comparable age were recruited from volunteers in the community and hospital staff. Exclusion criteria were blood transfusion within the previous 3 months, malignancy, connective tissue disease, cardiovascular disease, diabetes, hypertension, pregnancy or taking warfarin or long-term medication such as hydroxyurea or oral contraceptives. None of the patients had a painful crisis within 1 month of the blood sampling. The approval of Ethics Committee of West Birmingham Health Authority and written informed consent from all participants was obtained.

Venepuncture was performed in the morning between 9 AM and 12 Noon on subjects following a minimum of 20 min in the supine position. Venous blood was taken into 3.2% sodium citrate for sP-selectin and pP-selectin, and into citrate, theophylline, adenosine and dipyridamole (CTAD) for

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