Platelet hyperactivity in sickle-cell disease: a consequence of hyposplenism

MW KENNY, AJ GEORGE, AND J STUART

From the Department of Haematology, University of Birmingham and the Department of Haematology, Queen Elizabeth Hospital, Birmingham B15 2TH, UK

SUMMARY Platelet function was measured on 29 occasions in 16 adult patients in the asymptomatic steady state of sickle-cell anaemia. There was a significant increase in platelet number and micro-aggregate formation, and a lower aggregation threshold with adenosine diphosphate, compared with 23 healthy controls. Similar changes were found, however, in 12 splenectomised patients without sickle-cell disease. The platelet hyperactivity of the sickle-cell steady state therefore reflects an increased circulating population of young, metabolically active platelets resulting from previous autosplenectomy.

Although the irreversibly sickled cell is the major cause of vascular obstruction in the painful vaso-occlusive crisis of sickle-cell disease, there is evidence that platelets are also involved. Liver biopsy during sickle-cell crisis has revealed platelet masses in association with aggregates of sickled cells,¹ the platelet count may fall during crisis compared with steady-state values,^{2,3} and there is a reduction in platelet survival.⁴ Clinical recovery is associated with a rebound thrombocytosis.^{3,5}

It is important to determine whether these changes are secondary to vascular stasis and endothelial damage during crisis⁶ or whether there is pre-existing platelet activation during the steady state which contributes to the onset of vaso-occlusive crisis. Published reports of platelet function in asymptomatic sickle-cell disease have given conflicting results: platelet aggregability has been reported as increased,⁷ normal,^{4,8} and decreased,⁹ with either no change⁴ or decreased aggregability^{8,9} during crisis. There is, however, one report of aspirin-dipyridamole prophylaxis causing a small reduction in symptomatic episodes in three patients.¹⁰

The present study was designed to examine platelet function during the steady state in comparison with healthy controls and splenectomised patients.

Patients and methods

Blood samples were obtained, without venostasis,

from 16 patients with sickle-cell disease (12 S/S, three S/ β thalassaemia, and one S/C disease) of mean age 20.7 (range 15-42) years at routine outpatient visits. Each patient was studied on an average of two occasions in the steady state and had been asymptomatic for at least four weeks before testing; it has been shown that the haemostatic abnormalities, including rebound thrombocytosis, of sickle-cell crisis return to normal within four weeks.⁵ None of the patients had palpable splenomegaly. Blood was obtained under identical conditions from a control group of 23 healthy subjects of mean age 24.7 (range 19-30) years. The tests which showed a statistically significant difference between these two groups (see Table) were subsequently performed on blood from 12 splenectomised patients without sickle-cell disease of mean age 19.9 (range 7-33) years. The splenectomy had previously been performed, as part of the staging procedure for lymphoma, at least three months previously, and the patients were not currently receiving cytotoxic drugs or radiotherapy. All the patients had also avoided, for at least two weeks, any drugs that interfere with platelet function.

Platelet counts, using EDTA-anticoagulated blood, were performed using the Thrombofuge-Thrombocounter system (Coulter Electronics Ltd). Blood for platelet function studies was collected into 1/10th volume of 3.13% trisodium citrate in polystyrene tubes and centrifuged once at 22°C and 200 g for 10 minutes, to produce platelet rich plasma (PRP), and then for 15-20 minutes at 22°C and 1500 g, to obtain platelet-poor plasma (PPP). The platelet counts of PRP were adjusted to $300 \times 10^9/l$

Received for publication 15 October 1979

Table Mean ($\pm$ SD) results for the sickle-cell steady state compared with healthy controls and postsplenectomy patients

	Healthy controls		Sickle-cell steady state		Postsplenectomy
Platelet count ($\times 10^9/l$)	(23)* 232 ± 68		(29) 388 ± 141		(12) 465 ± 197
P		< 0.001		NS	
In-vivo microaggregate ratio	(21) 0.88 ± 0.13		(23) 0.68 ± 0.22		(12) 0.62 ± 0.23
P		< 0.001		NS	
ADP-threshold (mmol/l)	(23) 1.77 ± 0.80		(29) 1.36 ± 0.71		(12) 1.37 ± 1.18
P		< 0.05		NS	
Spontaneous aggregation (%)	(23) 6.2 ± 4.8		(29) 6.6 ± 4.7		
P		NS			
Heparin thrombin clotting time (s)	(23) 30.1 ± 12.8		(29) 36.6 ± 20.3		
P		NS			

*Number of tests in parentheses.

using autologous PPP, kept in capped polystyrene tubes at room temperature, and tested within 45 minutes.

Spontaneous platelet aggregation was performed by a modification of the method of Wu and Hoak¹¹ using 0.5 ml PRP mixed for 10 minutes at 700 rpm and at 37°C in a dual-channel platelet aggregometer (Malin Electronics Ltd, Ayr, Scotland) attached to a Vitatron 2001 recorder (MSE-Fisons Ltd, Crawley, Sussex). Transmission was adjusted to 0% for PRP and to 100% for PPP. The change in transmission was recorded as percentage spontaneous aggregation.

The minimum adenosine diphosphate (ADP) concentration required to produce a biphasic aggregation response (ADP threshold) was determined by adding 0.1 ml ADP (BDH Chemicals Ltd, Poole, Dorset) to 0.5 ml PRP to give final concentrations ranging from 0.2 to 5.5 mmol/l PRP.

Platelet *in vivo* microaggregate ratios were measured by the formalin-fixation method of Wu and Hoak¹² but using twice the volume of blood and buffer to improve accuracy.

The heparin thrombin clotting time (platelet factor 4-like activity) was determined according to O'Brien *et al.*¹³ using a thrombin concentration of 6 units/ml and adjusting the heparin concentration to give a control time of 25 seconds for pooled normal plasma.

Statistical significance was determined by the Mann-Whitney U test.

Results

Patients with sickle-cell disease, compared with

healthy controls, showed a significantly higher platelet count, lower *in vivo* microaggregate ratio, and lower ADP threshold (see Table). There was no significant difference in spontaneous aggregation or heparin thrombin clotting time.

When the results for sickle-cell patients were compared with those for postsplenectomy patients, there was no significant difference in platelet count, *in vivo* microaggregate ratio, or ADP threshold.

Discussion

This study of asymptomatic sickle-cell anaemia has shown a significant increase in platelet number and microaggregate formation and a lower threshold for ADP-induced aggregation, compared with healthy controls of similar age. The raised platelet count has previously been reported¹⁴⁻¹⁷ and an increase in large platelets (megathrombocytes) was also demonstrated in one study.¹⁶ It is particularly important to select appropriate control patients in studies of sickle-cell disease,¹⁸ and when we included a second control group of adult asplenic patients we could no longer demonstrate these differences in platelet number and function.

Functional asplenia, as determined by reduced uptake of isotope, develops in childhood sickle-cell disease¹⁹ and is followed by ischaemia-induced fibrotic atrophy.²⁰ Splenectomised adults, without sickle-cell disease, have a thrombocytosis^{21,22} caused by loss of the normal splenic platelet pool which contains up to one-third of the total body platelet mass.²³ Absence of this pool has also been demonstrated in sickle-cell anaemia.²⁴ The spleen has been

shown to retain newly formed platelets for up to two days before their release into the circulation²⁵ and to contain many large platelets²⁶ which are young,²⁷ more active metabolically,^{28, 29} and show greater responsiveness in platelet function tests.^{30, 31} The few studies of platelet function after splenectomy have shown normal adhesiveness^{32, 33} but increased aggregation with ADP and also collagen.³³

Thus the apparent platelet hyperactivity of the sickle steady state reflects the absence of splenic pooling of young active platelets rather than chronic intravascular activation of platelets in the micro-circulation. Although a recent study of 318 post-splenectomy patients showed no significant association between postsplenectomy thrombocytosis and thromboembolism,³⁴ the increased number of circulating active platelets in sickle-cell disease may readily lead to aggregation in areas of stasis during vaso-occlusive crisis. This is consistent with the fall in platelet count,^{2, 3} reduction in platelet survival,⁴ and decrease in aggregability of the remaining circulating platelets.^{8, 9}

Platelet hyperactivity in the steady state, and also platelet activation during crisis, now appear to be secondary phenomena, and it seems unlikely that antiplatelet therapy could have a major effect in reducing the frequency or severity of sickle-cell crises.

We are indebted to Mr K Matthews, Department of Hospital Statistics, Queen Elizabeth Medical Centre, for help with the statistical analysis and to Dr KE Barrett and Dr JR Mann for referring patients.

References

- Rosenblate HJ, Eisenstein R, Holmes AW. The liver in sickle cell anemia: a clinical-pathologic study. *Arch Pathol* 1970;**90**:235-45.
- Gordon PA, Breeze GR, Mann JR, Stuart J. Coagulation fibrinolysis in sickle-cell disease. *J Clin Pathol* 1974;**27**:485-9.
- Ittyerah R, Alkjaersig N, Fletcher A, Chaplin H. Coagulation factor XIII concentration in sickle-cell disease. *J Lab Clin Med* 1976;**88**:546-54.
- Haut MJ, Cowan DH, Harris JW. Platelet function and survival in sickle cell disease. *J Lab Clin Med* 1973;**82**:44-53.
- Alkjaersig N, Fletcher A, Joist H, Chaplin H, Jr. Hemostatic alterations accompanying sickle cell pain crises. *J Lab Clin Med* 1976;**88**:440-9.
- Rickles FR, O'Leary DS. Role of coagulation system in pathophysiology of sickle cell disease. *Arch Intern Med* 1974;**133**:635-41.
- Barnhart MI, Vettrano A, Lusher JM. Microscopy as an aid in the evaluation and management of sickle cell anemia. *Thromb Diath Haemorrh* 1973;Suppl 53: 193-214.
- Stuart MJ, Stockman JA, Oski FA. Abnormalities of platelet aggregation in the vaso-occlusive crisis of sickle-cell anemia. *J Pediatr* 1974;**85**:629-32.
- Gruppo RA, Glueck HI, Granger SM, Miller MA. Platelet function in sickle cell anemia. *Thromb Res* 1977;**10**:325-35.
- Alkjaersig N, Chaplin H, Fletcher AP. Experience with aspirin-dipyridamole prophylaxis in sickle cell disease. *Thromb Haemost* 1979;**42**:60. (Abstract)
- Wu KK, Hoak JC. Spontaneous platelet aggregation in arterial insufficiency: mechanisms and implications. *Thromb Haemost* 1976;**35**:702-11.
- Wu KK, Hoak JC. A new method for the quantitative detection of platelet aggregates in patients with arterial insufficiency. *Lancet* 1974;**2**:924-6.
- O'Brien JR, Etherington M, Jamieson S, Lawford P. Heparin thrombin clotting-time and platelet factor 4. *Lancet* 1974;**2**:656-7. (Letter)
- Green D, Kwaan HC, Ruiz G. Impaired fibrinolysis in sickle cell disease. Relation to crisis and infection. *Thromb Diath Haemorrh* 1970;**24**:10-6.
- Walsh RT, Lusher JM, Barnhart MI. Coagulation and fibrinolysis studies in sickle cell anemia. *Thromb Diath Haemorrh* 1973;Suppl 53:271-9.
- Freedman ML, Karparkin S. Short communication: elevated platelet count and megathrombocyte number in sickle cell anemia. *Blood* 1975;**46**:579-82.
- Leslie J, Langer D, Serjeant GR, Serjeant BE, Desai P, Gordon YB. Coagulation changes during the steady state in homozygous sickle-cell disease in Jamaica. *Br J Haematol* 1975;**30**:159-66.
- Richardson SGN, Matthews KB, Stuart J, Geddes AM, Wilcox RM. Serial changes in coagulation and viscosity during sickle-cell crisis. *Brit J Haematol* 1979;**41**:95-103.
- Pearson HA, Spencer RP, Cornelius EA. Functional asplenia in sickle cell anemia. *N Engl J Med* 1969;**281**:923-6.
- Diggs LW. Siderofibrosis of the spleen in sickle cell anemia. *JAMA* 1935;**104**:538-41.
- Singer K, Miller EB, Dameshek W. Hematologic changes following splenectomy in man, with particular reference to target cells, hemolytic index and lysolecithin. *Am J Med Sci* 1941;**202**:171-87.
- Lipson RL, Bayrd ED, Watkins CH. The postsplenectomy blood picture. *Am J Clin Pathol* 1959;**32**:526-32.
- Aster RH. Pooling of platelets in the spleen: Role in the pathogenesis of 'hypersplenic' thrombocytopenia. *J Clin Invest* 1966;**45**:645-57.
- Schwartz AD. The splenic platelet reservoir in sickle cell anemia. *Blood* 1972;**40**:678-83.
- Shulman NR, Watkins SP, Jr, Itscoitz SB, Students AB. Evidence that the spleen retains the youngest and hemostatically most effective platelets. *Trans Assoc Am Physicians* 1968;**81**:302-13.
- Freedman ML, Karparkin S. Heterogeneity of rabbit platelets. V. Preferential splenic sequestration of megathrombocytes. *Br J Haematol* 1975;**31**:255-62.
- Amorosi E, Garg SK, Karparkin S. Heterogeneity of human platelets. IV. Identification of a young platelet population with (⁷⁵Se) selenomethionine. *Brit J Haematol* 1971;**21**:227-32.

- ²⁸ Karparkin S, Charvatz A. Heterogeneity of human platelets. I. Metabolic and kinetic evidence suggestive of young and old platelets. *J Clin Invest* 1969;48: 1073-82.
- ²⁹ Karparkin S, Charvatz A. Heterogeneity of human platelets. III. Glycogen metabolism in platelets of different sizes. *Brit J Haematol* 1970;19:135-43.
- ³⁰ Mannucci PM, Sharp AA. Platelet volume and shape in relation to aggregation and adhesion. *Brit J Haematol* 1967;13:604-17.
- ³¹ Karparkin S. Heterogeneity of human platelets. II. Functional evidence suggestive of young and old platelets. *J Clin Invest* 1969;48:1083-7.
- ³² McClure PD, Ingram GIC, Stacey RS, Glass UH, Matchett MO. Platelet function tests in thrombocythaemia and thrombocytosis. *Brit J Haematol* 1966;12:478-98.
- ³³ Zucker S, Mielke CH. Classification of thrombocytosis based on platelet function tests: Correlation with hemorrhagic and thrombotic complications. *J Lab Clin Med* 1972;80:385-94.
- ³⁴ Boxer MA, Braun J, Ellman L. Thromboembolic risk of postsplenectomy thrombocytosis. *Arch Surg* 1978; 113:808-9.

Requests for reprints to: Professor J Stuart, Queen Elizabeth Hospital, Birmingham B15 2TH, UK.

Reports and Bulletins prepared by the Association of Clinical Biochemists

The following reports and bulletins are published by the Association of Clinical Biochemists. They may be obtained from The Publishing Department, British Medical Journal (ACB Technical Bulletins), BMA House, Tavistock Square, London WC1H 9JR. Overseas readers should remit by British Postal or Money Order.

SCIENTIFIC REVIEWS (price £1.00/\$2.00 each)

- 1 The assessment of thyroid function March 1971
FV FLYNN and JR HOBBS
- 2 Renal function tests suitable for clinical practice
January 1972 FL MITCHELL, N VEALL, and RWE WATTS
- 3 Biochemical tests for the assessment of fetoplacental
function May 1975 CE WILDE and RE OAKEY
- 4 Test of exocrine pancreatic function March 1977
AH GOWENLOCK
- 5 Assay of cholinesterase in clinical chemistry March
1979 ELSIE SILK, J KING, and MARY WHITTAKER

TECHNICAL BULLETINS (price £1.00/\$2.00 each)

- 22 Bilirubin standards and the determination of bilirubin
by manual and technicon AutoAnalyzer methods
January 1971 BARBARA BILLING, RUTH HASLAM, and N
WALD
- 23 Interchangeable cells for spectrophotometers and
fluorimeters September 1971 SS BROWN and AH
GOWENLOCK
- 24 Simple tests to detect poisons March 1972 BW
MEADE *et al.*
- 25 Blood gas analysers May 1972 K DIXON
- 26 Kits for enzyme activity determination September
1972 SB ROSALKI and D TARLOW
- 27 Assessment of pumps suitable for incorporation into
existing continuous flow analytical systems November
1972 A FLECK *et al.*
- 28 Routine clinical measurements of transferrin in
human serum September 1973 K DIXON

- 29 Control materials for clinical biochemistry (5th
edition) September 1973 JF STEVENS

- 30 Notes on the quality of performance of serum
cholesterol assays September 1973 SS BROWN

- 31 Determination of uric acid in blood and in urine
July 1974 RWE WATTS

- 32 A survey of amino acid analysers readily available in
the United Kingdom September 1974 JE CARLYLE
and P PURKISS

- 33 Definitions of some words and terms used in auto-
mated analysis November 1974 A FLECK, R ROBINSON,
SS BROWN, and JR HOBBS

- 34 Measurement of albumin in the sera of patients
January 1975 LINDA SLATER, PM CARTER, and JR HOBBS

- 35 Investigation of the validity of temperature correction
factors for serum aspartate and alanine transaminases
March 1975 SB ROSALKI *et al.*

- 36 Factors influencing the assay of creatinine November
1975 JGH COOK

- 37 A survey of enzyme reaction rate analysers readily
available in the United Kingdom July 1977 RA
SAUNDERS and RF BURNS

- 38 Transport of specimens for clinical chemistry an-
alysis November 1977 P WILDING, JF ZILVA, and
CE WILDE

- 39 A scheme for the evaluation of diagnostic kits May
1978 PH LLOYD

- 40 A practical guide to gamma-counting in radio-
immunoassay January 1980 CE WILDE and D OTTEWELL