



Hypercoagulability in Sickle Cell Disease: New Approaches to an Old Problem

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Patients with sickle cell disease (SCD) exhibit high plasma levels of markers of thrombin generation, depletion of natural anticoagulant proteins, abnormal activation of the fibrinolytic system, and increased tissue factor expression, even in the non-crisis steady state. In addition, platelets and other cellular elements are chronically activated in the non-crisis state. Despite an abundance of evidence for coagulation and platelet activation, it remains uncertain whether these changes contribute to the pathophysiology of SCD or

are, rather, simple epiphenomena. With the occurrence of macrovascular thrombotic complications in SCD, as well as the recognition that soluble CD40 ligand is biologically active in SCD, coagulation and platelet activation may indeed play a role in SCD pathophysiology. Defining a role for hypercoagulability in SCD requires further understanding of its pathogenesis. Furthermore, the conduct of well-controlled clinical trials using anticoagulants and antiplatelet agents and using a variety of clinical endpoints is warranted.

Introduction

Sickle cell disease (SCD), an inherited disorder characterized by the presence of sickle hemoglobin (HbS), results from the substitution of glutamic acid by valine at the 6th position of the β -globin chain. In addition to the well-known hemolytic and vaso-occlusive complications, patients with SCD are at risk for a variety of thrombotic complications. Ischemic stroke, caused by large vessel arterial obstruction with superimposed thrombosis, occurs commonly in patients with SCD.¹ New and old thrombi in the pulmonary vasculature are prevalent in autopsy series.² A recent retrospective study based on reported discharge diagnoses showed that patients with SCD younger than 40 years were more likely to be diagnosed with pulmonary embolism compared with African Americans without SCD (0.44% vs 0.12%), although, interestingly, the prevalence of deep vein thrombosis was similar between the two groups.³ SCD also appears to be a significant risk factor for pregnancy-related venous thromboembolism, with an odds ratio of 6.7 (95% confidence interval: 4.4-10.1).⁴ Finally, although clearly distinct from SCD in its clinical manifestations, carriers of the sickle cell trait appear to be at increased risk of venous thromboembolism compared to race-matched controls, with an odds ratio of about 2.⁵ Of interest in this regard, sickle cell trait appeared to be associated with pulmonary embolism rather than deep vein thrombosis,⁵ analogous to the situation in patients with SCD.³

Pathogenesis

Nearly every component of hemostasis, including platelet function and the procoagulant, anticoagulant, and fibrinolytic systems, is altered in the direction of a procoagulant phenotype in this disease. As a result of these findings, SCD is frequently referred to as a "hypercoagulable state."⁶ Loss of normal membrane phospholipid asymmetry is present in a subpopulation of red blood cells (RBC) in

SCD patients, occurring in mature cells, RNA-containing reticulocytes, and transferrin receptor-positive "stress" erythrocytes.^{7,8} Normally, phosphatidylserine (PS) is found in the inner monolayer of the cell membrane, whereas choline-containing phospholipids such as phosphatidylcholine and sphingomyelin are located in the outer monolayer in the plasma membrane.⁹ RBC membrane phospholipid asymmetry is usually maintained by the action of an ATP-dependent aminophospholipid translocase (or flipase) that transports PS and phosphatidylethanolamine from the outer to the inner membrane surface.⁹ In addition, scramblase, when activated by an influx of extracellular calcium, results in the movement of all phospholipids in both directions, resulting in rapid PS exposure.¹⁰ Abnormal PS exposure in sickle erythrocytes may occur due to repeated cycles of sickling and unsickling linked to polymerization and depolymerization of HbS that results in the production of terminal spicules or microvesicles with exposed PS.¹¹ The abnormal PS exposure may also result from reduced flipase activity in RBC due to oxidative stress and sulphhydryl modification.^{9,12,13} This abnormal PS exposure functions as both a recognition signal for cell removal during apoptosis of nucleated cells¹⁴ and a docking site for enzymatic complexes involved in coagulation and anticoagulation pathways.¹⁰ External exposure of PS alters the adhesive properties of sickle RBC¹⁵ and appears to be involved in the hemostatic changes observed in SCD.^{16,17} In addition to its possible contribution to an increased risk of stroke in patients with SCD,¹⁸ several pieces of data provide evidence for a relationship between PS exposure and the hemostatic changes. First, plasma levels of prothrombin fragment 1.2 (F1.2) are associated with the number of circulating PS-positive RBC.¹⁷ However, although the number of PS-positive sickle RBC is significantly correlated with plasma F1.2, D-dimer, and plasmin-antiplasmin (PAP) complexes, no correlation is found be-

tween PS-positive platelets and any of these hemostatic markers,¹⁶ suggesting that sickle RBC, and not platelets, are responsible for the hemostatic activation observed in SCD. Finally, sickle RBC containing high amounts of fetal hemoglobin (HbF) are less likely to result in microparticle formation, PS exposure, and thrombin generation,¹⁷ suggesting a protective effect of HbF on membrane bilayer flip-flop and PS exposure.

A recent study demonstrated that type II PS RBC (highly PS-positive and including dense sickle erythrocytes) cause a twofold increase in endothelial tissue factor (TF) expression *in vitro*, an effect that may not be due to a direct physical interaction of red cells with the endothelium in patients with SCD,¹⁹ but rather is a result of increased circulating hemoglobin resulting from hemolysis. The greater correlations observed between type II PS RBC and both circulating whole blood TF and plasma cell-free hemoglobin compared with the type I RBC fraction (containing erythrocytes with a relatively low level of PS expression, and including a majority of reticulocytes) suggest that increased hemolysis of type II PS-positive RBC may contribute to the hypercoagulability in patients with SCD (**Figure 1**; see Color Figures, page 520).

Many of the same coagulation changes that have been described in SCD, such as increased plasma levels of thrombin-antithrombin (TAT) complexes, F1.2, and D-dimers, have also been reported in patients with sickle cell trait, suggesting that a direct link may exist between carriage of the β^s gene and activation of the coagulation system.²⁰

Finally, the inflammatory state in SCD may also contribute to hypercoagulability. Studies in transgenic mice demonstrate that exposure of the mild sickle-cell phenotype, NY1DD mice, to a hypoxic environment for 3 hours followed by a return to ambient air for 18 hours resulted in increased TF expression in the pulmonary veins. Thus, ischemia-reperfusion injury in patients with SCD may play a pathophysiologic role in the activation of coagulation.²¹

Increased Tissue Factor Expression

The TF-FVIIa complex is the physiologic initiator of hemostasis.²² Abnormal expression of TF on circulating endothelial cells has been demonstrated in patients with SCD, and its expression is increased further during pain episodes.²³ In addition, both TF antigen and TF procoagulant activity are elevated in the circulation of patients with SCD when compared with healthy control participants,²⁴⁻²⁶ although no difference in whole-blood TF procoagulant activity was observed in patients in the non-crisis steady state compared with those with pain crises.²⁴

While numerous plasma factors that could increase TF expression in hematopoietic and/or endothelial cells, such as thrombin, interleukin-1, tumor necrosis factor, and endotoxin, are elevated in patients with SCD, multiple potential mechanisms for the increased TF expression in SCD have recently been described. As already described, these include ischemia-reperfusion injury²¹ and increased hemo-

lysis of type II PS-positive cells,¹⁹ as well as induction of TF expression in monocytes by increased plasma levels of soluble CD40 ligand (sCD40L).²⁶

Alteration in Markers of Thrombin Generation and Anticoagulant Proteins

Patients with SCD have increased plasma levels of markers of thrombin generation in the non-crisis steady state, although there are conflicting data on the effect of acute pain episodes on the levels of these markers.^{27,28} Plasma levels of F1.2 and TAT complexes, both markers of thrombin generation, are elevated in patients with SCD in the non-crisis steady state.^{27,28} Similar elevations in plasma levels of D-dimers,^{25,27,28} PAP complexes,²⁷ and fibrinopeptide A²⁸ are observed in patients with SCD in the non-crisis, steady state. The frequency of pain episodes in patients with SCD correlates with the extent of fibrinolytic activity (assessed by D-dimer levels) in the non-crisis steady state, suggesting that D-dimer levels may predict the frequency of pain crises.²⁷

Decreased levels of natural anticoagulant proteins are also observed in SCD. Thus, levels of protein C and protein S are decreased^{28,29} in the non-crisis steady state and perhaps even more so during acute pain episodes.^{28,30} Reduced levels of these regulatory proteins may be a consequence of (1) chronic consumption due to increased thrombin generation resulting from intravascular TF expression and RBC prothrombinase activity; (2) increased binding of protein S by sickle RBC due to membrane PS exposure³¹; and/or (3) inhibition of the binding of protein S to β_2 -glycoprotein 1 by antiphospholipid antibodies,³² resulting in inactivation of protein S by circulating C4b-binding protein. Significantly decreased levels of proteins C and S were reported in patients with SCD who developed thrombotic strokes compared with neurologically normal children with SCD.³³ Plasma levels of the serine protease inhibitor (SERPIN) heparin cofactor II (HCII) are also decreased in SCD.³⁴

Antiphospholipid antibodies are detected with relatively high frequency in patients with SCD.²⁸ In addition, levels of certain sub-types of antiphospholipid autoantibodies, particularly those directed against PS, are markedly elevated in patients with homozygous SCD (HbSS), whereas they are normal in patients with HbSC disease.²⁸ In patients with HbSS, a strong correlation exists between antibodies against PS and plasma D-dimers, suggesting a significant role for anti-PS antibodies in coagulation activation in SCD.²⁸

Platelets, Endothelial Cells and Microparticles

There is an abundance of evidence suggesting that circulating platelets in patients with SCD are chronically activated. This activation may contribute to the observed hypercoagulable state in SCD.²⁶ While there are conflicting reports of platelet survival,^{35,36} platelet aggregation responses do appear to be increased in adult patients with SCD in the non-crisis steady state.³⁷ This phenomenon may be explained by increased numbers of circulating young, metabolically active platelets, or increased plasma levels

of platelet agonists such as thrombin, adenosine diphosphate, and epinephrine. Flow cytometry studies show increased platelet expression of both CD62P (P-selectin) and CD40L in patients with SCD.^{27,38} In addition to elevated plasma levels of the α -granule constituents, thrombospondin, platelet factor 4, and β -thromboglobulin,²⁷ platelet-derived plasma sCD40L is also elevated in the non-crisis, steady state, compared to control patients.²⁶ A recent study suggests that hemolysis, with decreased bioavailability of NO, may contribute, at least in part, to the pathogenesis of platelet activation in SCD.³⁹

Decreases in platelet lifespan and platelet counts have been reported during acute pain episodes.^{35,36} Furthermore, acute pain episodes appear to be associated with increased platelet activation compared with the non-crisis steady state.^{26,27} These findings suggest that decreased platelet survival and increased consumption occur during acute pain episodes, a likely result of platelet deposition at sites of vascular injury.

Patients with SCD have been described to have increased numbers of circulating endothelial cells in the non-crisis steady state, with a further accentuation of numbers during acute pain episodes.⁴⁰ In addition to expressing adhesion markers such as intercellular adhesion molecule 1, vascular-cell adhesion molecule 1, E-selectin, and P-selectin, these cells abnormally express TF antigen in the steady state, and to a greater extent during acute pain episodes.²³ Furthermore, these circulating endothelial cells are positive for TF mRNA, with excellent correlation between TF antigen and mRNA expression.²³

Circulating microparticles (MP), small membrane-derived vesicles released by cells following activation or apoptosis, may be derived from RBC, platelets, endothelial cells and monocytes.⁴¹ Both the total number of MP as well as the number of TF-positive MP are increased in patients with SCD, with further increases during acute pain episodes. These TF-positive MP are derived from endothelial cells and monocytes, but not from RBC and platelets.⁴¹ Finally, plasma markers of coagulation activation, such as D-dimer, TAT, and F1.2, appear to correlate with total MP, total TF-positive MP, monocyte-derived TF-positive MP, and RBC-derived MP,⁴¹ suggesting a role for MP in the hypercoagulable state observed in patients with SCD.

Therapeutic Implications

Despite the abundant laboratory evidence of hypercoagulability observed in patients with SCD, clinical studies using anticoagulants and antiplatelet agents have not provided any convincing benefit in the prevention or treatment of vaso-occlusive complications. Although most of these studies have been small and/or poorly controlled, it still remains uncertain whether the observed platelet activation as well as increased thrombin and fibrin generation contribute to the vascular occlusive episodes that characterize SCD, or are simple epiphenomena.

Treatment modalities frequently used in SCD appear

to affect the hemostatic system in these patients. Prophylactic RBC transfusion significantly reduces the risk of strokes in children with sickle cell anemia.⁴² It has been proposed that the beneficial effect may in part be related to normalization of the levels of F1.2,¹⁸ although another study found no significant reduction in thrombin generation when children with SCD undergoing chronic transfusion were compared with control patients.⁴³ Treatment with both hydroxyurea and decitabine also decrease plasma markers of thrombin generation.^{44,45}

Antiplatelet agents

There are only a few reports on the use of antiplatelet agents in SCD^{35,46-49} (**Table 1**). However, most of these studies did not correlate the *in vivo* effect of the drugs on platelet activation with specific clinical endpoints. A randomized, double-blind, placebo-controlled study evaluating the effect of ticlopidine reported a reduction in the frequency, duration, and severity of acute pain episodes in patients with SCD on ticlopidine following 6 months of therapy.⁵⁰ The results of this study and recent reports that platelet-derived CD40L appears to have biological activity in SCD²⁶ suggest that antiplatelet agents, particularly when administered at doses sufficient to inhibit platelet activation and/or CD40L release, may be beneficial in preventing or treating vaso-occlusive complications in patients with SCD.

Anticoagulant agents

Although multiple studies using anticoagulants have been carried out in SCD, these studies have been small and/or uncontrolled⁵¹⁻⁵⁴ (**Table 2**). Low-intensity anticoagulation using acenocoumarol has been reported to normalize circulating markers of thrombin generation in patients with SCD,^{52,53} although no reduction in the frequency of pain episodes was observed.⁵³ However, minidose unfractionated heparin appears to decrease the frequency and severity of acute pain episodes.⁵⁴ In addition to decreasing thrombin generation, this clinical effect may be due, at least in part, to the observation that heparin decreases sickle cell adhesion to endothelium under static conditions, as well as P-selectin-mediated flow adherence of sickle cells to thrombin-treated human vascular endothelial cells.⁵⁵

Finally, all of the published studies of anticoagulants in SCD to date have used pain crises as the clinical endpoint. With the complex pathophysiology of acute pain episodes, clinical studies of anticoagulants in the treatment and/or prevention of SCD-related complications that are known to be associated with large vessel arterial and venous thrombosis are required.

Conclusions

The bulk of evidence demonstrates that there is increased platelet and plasma coagulation activation in SCD. Coagulation activation in patients with SCD appears to be a consequence of the exposure of PS on the outer surface of the RBC membrane, ischemia-reperfusion injury, and pos-

Table 1. Published studies of antiplatelet agents in patients with sickle cell disease (SCD).

Study	Genotype	No. of patients	Therapy	Randomized	Duration	Efficacy outcome measure and results
Chaplin et al ⁴⁷ (1980)	HbSS	3	Aspirin/Dipyridamole	No	104 weeks	Modest decrease in frequency of pain episodes, platelet count, and fibrinogen level
Osamo et al ^{48*} (1981)	HbSS	100	Aspirin	Yes	6 weeks	Increase in oxygen affinity, hemoglobin, and RBC life span
Greenberg et al ⁴⁶ (1983)	HbSS	40	Aspirin vs placebo	Yes	21 months	No decrease in frequency of pain episodes
	HbSC	8				
	HbS-O Arab	1				
Semple et al ³⁶ (1984)	HbSS	8	Ticlopidine vs placebo	Yes	4 weeks	No improvement in frequency of pain episodes or platelet survival, but decrease in platelet release products
	HbS-β thalassemia	1				
Cabannes et al ^{50†} (1984)	HbSS	140	Ticlopidine vs placebo	Yes	6 months	Reduction of frequency and duration of pain episodes
Zago et al ⁴⁹ (1984)	HbSS HbS-β thalassemia	25 4	Aspirin vs placebo	Yes	5 months	No differences in frequency of pain episodes, hemoglobin, reticulocyte count, irreversibly sickled cells, and fetal hemoglobin level

*Increase from baseline in hemoglobin (7.5 ± 0.35 g/dL to 10 ± 0.39 g/dL; $P < .01$), O₂ saturation ($80\% \pm 3.6\%$ to $94\% \pm 3.7\%$, $P < .01$), pO₂ (52 ± 2.7 mm Hg to 68 ± 2.9 mmHg; $P < .01$), and decrease in 2,3-diphosphoglycerate levels (7.2 ± 0.24 μmol/mL to 4.6 ± 0.20 μmol/mL, $P < .01$) in patients on aspirin after 6 weeks of treatment. No data provided for patients in the control group.

†Decrease in number of crises (-81% vs -47% ; $P = .0001$) and duration of crises (2.862 ± 1.75 days vs 3.952 ± 2.2 days; $P = .001$) in patients on ticlopidine vs placebo

Table 2. Published studies of anticoagulants in patients with sickle cell disease (SCD).

Study	Genotype	No. of patients	Therapy	Randomized	Duration	Efficacy outcome measure and results
Salvaggio et al ⁵¹ (1963)	HbSS	12	Warfarin	No	12-34 months	Modest decrease in frequency of pain episodes
Chaplin et al ⁵⁴ (1989)	HbSS	4	Heparin	No	2-6 years	Reduced frequency of pain episodes
Wolters et al ⁵² (1995)	HbSS	6	Acenocoumarol	No	2 months	Reduced prothrombin fragment 1.2
	HbSC	1				
Schnog et al ⁵³ (2001)	HbSS	14	Acenocoumarol vs placebo	Yes	14 weeks	Reduced markers of coagulation activation, but no reduction of pain episodes with active treatment
	Hb C	8				

sibly increased NO scavenging as a result of the increased fragility of type II PS-positive red cells.

It remains uncertain whether the coagulation and platelet activation observed in patients with SCD contribute to the pathophysiology of SCD or are secondary events. Defining the contribution of the hypercoagulable state to the pathophysiology of SCD requires further studies using transgenic animal models. Furthermore, with increasing evidence that NO plays a crucial role in the pathophysiology of SCD, further studies evaluating its contribution to the hypercoagulability observed in SCD are required. Finally, well-controlled clinical studies of anticoagulants and/or antiplatelet agents using a variety of clinical endpoints will help to further define the contribution of hypercoagulability to the pathophysiology of SCD and its complications.

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