β-Thalassaemia and sickle cell anaemia as paradigms of hypercoagulability

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Summary

Thalassaemia and sickle cell disease (SCD) represent the most common forms of hereditary haemolytic anaemia and result from a partial or complete lack of synthesis of one of the major α- or β-globin chains of haemoglobin A or from a single amino acid mutation ($\beta^{6Glu \rightarrow Val}$) of the β-globin chain respectively. Although they have different pathophysiologies, patients with these conditions manifest both biochemical and clinical evidence of hypercoagulability. While the frequency of various thrombotic complications may vary in β-thalassaemia and homozygous SCD [sickle cell anaemia (SCA)], patients with both diseases manifest decreased levels of natural anticoagulant proteins, as well as increased markers of thrombin generation and platelet activation. The abnormal phospholipid membrane assymetry present in the red blood cells of β-thalassaemia and SCA patients, with resultant phosphatidylserine exposure appears to play a significant role in the aetiology of the observed hypercoagulable state. This review presents the available data on the aetiology and clinical manifestations of the coagulation and platelet activation that exist in both β-thalassaemia and SCA, as well as the potential therapeutic implications resulting from this hypercoagulability.

Keywords: β-thalassaemia, sickle cell anaemia, thrombin generation, platelet activation, nitric oxide.

Thalassaemia and sickle cell disease (SCD) represent the most common genetic disorders worldwide (Angastiniotis & Modell, 1998; Higgs et al, 2001). Although they have different pathophysiologies, patients with both diseases share many clinical manifestations. In addition, these patients exhibit thrombotic complications, with several published series demonstrating the presence of both arterial and venous thromboses. Thrombotic stroke, caused by large vessel obstruction with superimposed thrombosis, occurs commonly in patients with SCD (Prengler et al, 2002). While less common in thalassaemia, stroke has also been reported in patients with β-thalassaemia major from Greece and Italy with an incidence of 20% and 2% respectively (Borgna-Pignatti et al, 1998), and in patients with β-thalassaemia/haemoglobin (Hb) E disease (Wong et al, 1990). This complication occurs more commonly in patients who are not regularly transfused. Evidence for asymptomatic brain damage has also been reported by magnetic resonance imaging in patients with β-thalassaemia intermedia and is inversely correlated with the Hb level and increasing age (Manfre et al, 1999). Autopsy series in SCD patients showed evidence of new and old thrombi in the pulmonary vasculature (Adedeji et al, 2001). In addition, several clinical series describe the occurrence of deep venous thrombosis (DVT), pulmonary embolism and portal vein thrombosis in patients with β-thalassaemia major and β-thalassaemia intermedia (van Teunenbroek et al, 1989; Michaeli et al, 1992; Gillis et al, 1999; Cappellini et al, 2000). A recent report based on data from the National Hospital Discharge Survey in the United States showed that SCD patients <40-years of age had a higher discharge diagnosis of pulmonary embolism compared with African Americans without SCD (0·44% vs. 0·12%), although the prevalence of DVT was similar (Stein et al, 2006). Furthermore, the Nationwide Inpatient Sample from the Healthcare Cost and Utilization Project of the Agency for Healthcare Research and Quality, from 2000 to 2001, reported that SCD is a significant risk factor for pregnancy-related venous thromboembolism, with an odds ratio of 6·7 (95% confidence interval: 4·4–10·1) (James et al, 2006). Finally, intimal hyperplasia is found in a variety of vascular beds including pulmonary, renal (Khademi & Marquis, 1973), splenic and placental vessels (Koshy et al, 1988) and in arterioles adjacent to leg ulcers (Serjeant, 1985) of SCD patients.

Unlike thalassaemia, most SCD-related complications are thought to be caused by microvascular occlusion and ischaemic tissue necrosis following the adhesion of erythrocytes (RBC) and other cellular elements to vascular endothelium, and the polymerization of sickle hemoglobin. However, recent evidence suggests that several complications in patients with

thalassaemia and SCD may share a similar pathogenesis. As a result of multiple clinical observations and laboratory data, both of these diseases have been referred to as 'hypercoagulable states' (Francis, 1991; Eldor & Rachmilewitz, 2002). With the heterogeneity observed in SCD and the various thalassaemic syndromes [as well as the limited data in sickle cell syndromes other than sickle cell anaemia (SCA)], this review will be restricted to a comparison between homozygous SCD (SCA) and β-thalassaemia. Furthermore, the role of antithrombotic therapy in these disorders will be discussed.

Pathogenesis

Red blood cell membrane alterations

Under normal conditions, the choline-containing phospholipids, phosphatidylcholine and sphingomyelin are mainly present in the outer monolayer of the plasma membrane, whereas phosphatidylserine (PS) is exclusively and phosphatidylethanolamine (PE) is mainly found in the inner monolayer (Zwaal & Schroit, 1997). It is accepted that this conformation is universal for all eukaryotic cell types and that PS is only exposed in situations that require a recognition signal for cell removal (Fadok et al, 1998), or for activation of certain reactions, such as the coagulation process (Schroit & Zwaal, 1991; Zwaal & Schroit, 1997). In normal RBC, maintenance of membrane phospholipid asymmetry appears to be provided by the action of an ATP-dependent aminophospholipid translocase (or flipase), that transports PS and PE from the outer to the inner membrane surface (Devaux & Zachowski, 1994), and a non-specific flopase that transports phospholipids from the inner to the outer monolayer (Bitbol & Devaux, 1988). In addition, a scramblase (a calcium-activated enzyme) results in the scrambling of all phospholipids to achieve rapid PS exposure (Zwaal & Schroit, 1997). Changes in the RBC membrane PS exposure are present in both β-thalassaemia and in SCA (Tait & Gibson, 1994) (Fig 1).

The membrane abnormalities of RBC from the patients with thalassaemia and SCA may result from excessive accumulation of oxidant damage. In thalassaemia, excess α- or β-globin chains are unstable and tend to oxidize and precipitate within
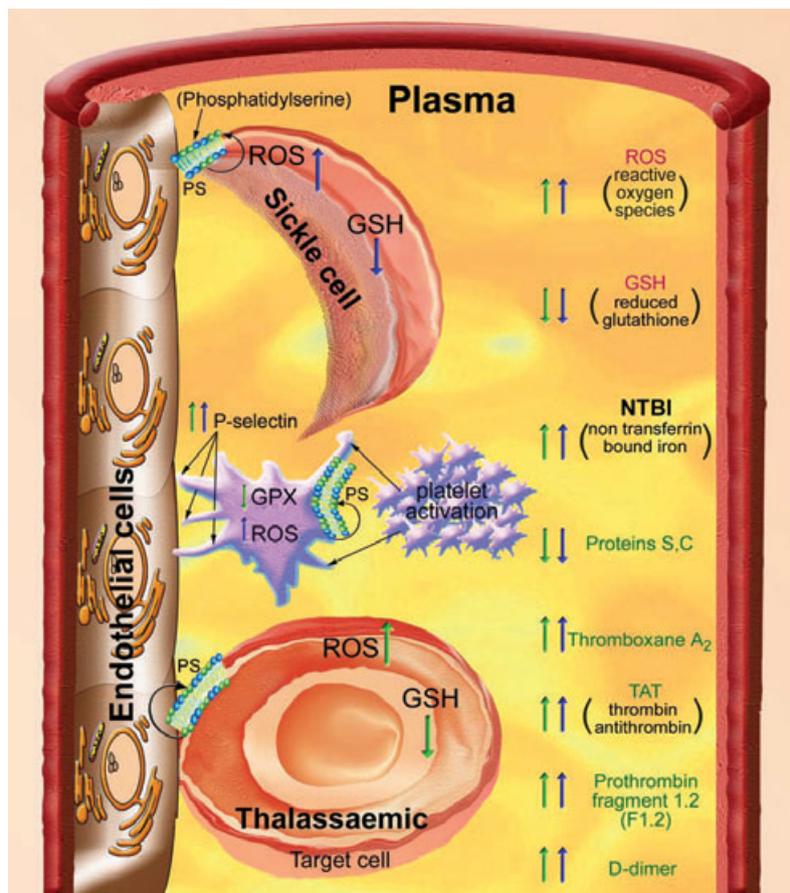

Fig 1. Pathophysiology of hypercoagulable state and platelet activation in thalassaemia and sickle cell disease (SCD) loss of the normal asymmetrical distribution of the RBC membrane phospholipids and translocation of phosphatidylserine to the external membrane leaflet (flip-flop) is present in thalassaemia and SCD, resulting in activation of the prothrombinase complex and facilitating interaction between the red blood cell and endothelial cells. The increase or decrease (double arrows) in all the plasma factors are described in the text or in Table I.

the RBC transforming to hemichromes (Rund & Rachmilewitz, 2005). Haem and the protein moiety eventually disintegrate, releasing toxic species of iron which catalyse the formation of reactive oxygen species (Livrea et al, 1996; Tavazzi et al, 2001), that in turn oxidize membrane lipids and result in the loss of membrane lipid organization (Kuypers & de Jong, 2004). Similar findings have been described in SCA (Hebbel et al, 1982).

Another factor which plays a role in the abnormal PS exposure in SCA appears to be the repeated cycles of sickling and unsickling that result from polymerization and depolymerization of sickle Hb. These cycles also lead to spicule and microvesicle formation, disruption of membrane phospholipid asymmetry and the exposure of PS in the outer monolayer of sickle RBC microvesicles (Allan et al, 1982). In addition, a striking correlation has been found between PS-positive sickle RBC and adhesion to vascular endothelium, suggesting an important contribution of these PS-positive cells to endothelial adhesion (Setty et al, 2000). The abnormal PS exposure on the outer monolayer of RBC in both SCA and β-thalassaemia results in the provision of a 'docking site' for both coagulation factor X and prothombinase complexes (Zwaal & Schroit, 1997), initiating the conversion of coagulation factor X to activated factor X and subsequent conversion of prothrombin to thrombin.

Although there are no studies in β-thalassaemia, a recent study of SCD patients suggests that type II PS RBC (highly PS-positive and including dense sickle cells) cause a twofold increase in endothelial tissue factor (TF) expression in vitro, an effect that may not be due to the physical interaction of the cells with the endothelium in these patients (B. N. Y. Setty, unpublished observations). Type II PS RBC appear to have a greater osmotic fragility compared with PS-negative RBC. Furthermore, greater correlations were observed between type II PS RBC and both whole blood TF and plasma cell-free Hb compared with type I PS RBC (relatively low levels of PS and including a majority of reticulocytes). In addition, a positive correlation was found between cell-free Hb and whole blood TF activity (B. N. Y. Setty, unpublished observations), suggesting that increased haemolysis of type II PS-positive RBC may contribute to the hypercoagulability in SCA patients.

Role of splenectomy/functional asplenia

It has been suggested that the absence of the spleen in a variety of haematological diseases may contribute to an increased propensity to thromboembolic complications (Cappellini et al, 2005). Both β-thalassaemia and SCA have a high proportion of patients who have no splenic function, either because of surgical splenectomy or because of functional hyposplenism. In a series of 83 patients with β-thalassaemia intermedia followed for 10 years, 29% developed pulmonary embolism, DVT of the lower extremities and portal vein thrombosis, and all but one patient had been splenectomized (Cappellini et al, 2000). The development of these complications has been attributed to the presence of high platelet counts following splenectomy in these patients (Eldor & Rachmilewitz, 2002). More recently, a multicentre study, conducted to assess the magnitude of thrombotic risk in β-thalassaemia patients, reported that 146 (1·65%) of 8860 patients experienced thrombotic events, with a prevalence of 0·9% in β-thalassaemia major and 4% in β-thalassaemia intermedia (Tahar et al, 2006). The highest prevalence of these events was observed in splenectomized patients. The occurrence of more frequent thrombotic events in β-thalassaemia patients who were not receiving regular transfusions (thalassaemia intermedia or thalassaemia major patients in less developed countries with limited transfusion resources) and in those patients who had undergone splenectomy strongly supports a procoagulant activity of circulating damaged RBC (Cappellini et al, 2000).

Moderate thrombocytosis has been reported in older children and adults with SCA (Haut et al, 1973; Freedman & Karpatikin, 1975), a probable consequence of the loss of splenic sequestration that follows autosplenectomy in these patients. However, the literature on the relationship between splenectomy (or autosplenectomy) and thrombosis in SCA is scant. Although treatment with hydroxycarbamide (also known as hydroxyurea) has been reported to decrease the risk of stroke in SCA patients (Ware et al, 2004; Gulbis et al, 2005), and may be associated with a lower prevalence of pulmonary hypertension in SCD patients (Ataga et al, 2004), there is no evidence that recovery of splenic function or platelet reduction following hydroxycarbamide treatment plays a role in these clinical scenarios.

While splenectomy, with subsequent thrombocytosis, may contribute to the development of thromboembolic phenomena in patients with β-thalassaemia, the increased number of circulating abnormal RBC following splenectomy, with resultant activation of platelets and the coagulation system, may be a more important contributor to the observed hypercoagulable state. This assumption is supported by the fact that repeated transfusions in both diseases significantly decrease the risk of thrombotic complications (Adams et al, 1998; Cappellini et al, 2000).

Inflammation

Although there are no data in patients with β-thalassaemia, the inflammatory state present in SCA may also contribute to the hypercoagulability observed in these patients. Studies in transgenic mouse models show that exposure of NY1DD mice (a mild sickle cell phenotype) to hypoxia-reoxygenation, by first placing them in a hypoxic environment for 3 h followed by a return to ambient air for 18 h, results in an increased expression of TF in their pulmonary veins (Solovey et al, 2004). This finding suggests a role for ischaemia-reperfusion injury in the pathogenesis of coagulation activation in SCA.

Platelet activation

There is an abundance of evidence suggesting that patients with both β-thalassaemia and SCA have activated platelets (Table I). This platelet activation may contribute to the observed hypercoagulable state. In both diseases, there is evidence for increased platelet aggregation (Kenny et al, 1980; Winichagoon et al, 1981; Westwick et al, 1983), although in SCA this observation has been described only in the adult patients. The normal or reduced platelet aggregation observed in children with SCA may be related to the fewer numbers of circulating young, large, and presumably more metabolically active platelets observed in these patients (Mehta & Mehta, 1980). Shortened platelet survival has been reported in patients with β-thalassaemia (Eldor et al, 1989) because of enhanced platelet consumption, although the data in SCA patients are inconsistent (Haut et al, 1973; Semple et al, 1984).

Flow cytometry studies demonstrate the presence of an increased fraction of platelets expressing the activation markers, CD62P (P-selectin) and CD63 in both SCA (Wun et al, 1998) and β-thalassaemia (DelPrincipe et al, 1993; Ruf et al, 1997). Elevated plasma levels of platelet factor 3 are found in patients with β-thalassaemia and SCA (Bunyaratvej, 1993; Famodu & Oduwa, 1995), and elevated plasma levels of both platelet factor 4 and β-thromboglobulin are observed in SCA patients (Tomer et al, 2001). In addition, platelet-derived soluble CD40 ligand (sCD40L) is elevated in SCA patients compared with normal controls (Lee et al, 2006). Increased binding of annexin-V to PS-rich platelet membrane domains have been found in SCA, similar to findings in β-thalassaemia (Ruf et al, 1997; Tomer et al, 2001). Finally, chronic-enhanced production of thromboxane A2 and prostaglandin metabolites have also been observed in the urine of patients with both β-thalassaemia and SCA because of chronic endogenous platelet activation (Eldor et al, 1991, 1993; Foulon et al, 1993).

Alterations in markers of coagulation activation and natural anticoagulant proteins

Prothrombin fragment 1.2 (F1.2), a marker of thrombin generation, is elevated both in SCA patients in the non-crisis, steady state (Kurantsin-Mills et al, 1992; Westerman et al, 1999) and in β-thalassaemia intermedia (Cappellini et al, 2000). It is significantly associated with PS-positive RBC in patients with SCA, providing evidence for the role of PS exposure in coagulation activation (Setty et al, 2001). Similar elevations in plasma levels of thrombin–antithrombin complexes and D-dimer, a marker of increased fibrinolysis, are observed in both diseases (Kurantsin-Mills et al, 1992; Eldor et al, 1999; Westerman et al, 1999; Cappellini et al, 2000; Setty et al, 2001).

Decreased levels of natural anticoagulant proteins are also observed in both SCA and β-thalassaemia. Levels of protein C and protein S are decreased in patients with SCA (Wright et al, 1997a; Eldor et al, 1999; Westerman et al, 1999) and β-thalassaemia (Visudhiphan et al, 1994; Eldor et al, 1999; Cappellini et al, 2000). The reduced levels of these anticoagulant proteins may be the result of chronic consumption because of an increase in the TF expression and thrombin generation and/or hepatic dysfunction (Musumeci et al, 1987; Wright et al, 1997a; Bayazit & Kilinc, 2001). Significantly decreased levels of these two anticoagulant proteins have been found in SCA patients who developed thrombotic strokes compared with neurologically normal SCA children (Tam, 1997). Heparin co-factor II (HCII), a circulating inhibitor of thrombin via a target enzyme specificity, which has similar

Table I. Evidence for increased platelet activation in β-thalassaemia and sickle cell anaemia (SCA) patients.

Platelet parameter	β-Thalassaemia	Sickle cell anaemia	References
Platelet aggregation	Increased	Increased	Kenny et al (1980); Winichagoon et al (1981); Westwick et al (1983)
Platelet survival	Decreased	Inconsistent results	Haut et al (1973); Semple et al (1984); Eldor et al (1989)
Plasma beta thromboglobulin	N/A	Increased	Tomer et al (2001)
CD62P (P-selectin) expression	Increased	Increased	DelPrincipe et al (1993); Ruf et al (1997); Wun et al (1998); Inwald et al (2000)
CD63 expression	Increased	Increased	Ruf et al (1997); Wun et al (1998)
Plasma platelet factor 4	N/A	Increased	Tomer et al (2001)
Plasma platelet factor 3	Increased	Increased	Bunyaratvej (1993); Famodu and Oduwa (1995)
Platelet CD40 ligand expression	N/A	Increased	Inwald et al (2000)
Plasma CD40 ligand	N/A	Increased	Lee et al (2006)
Membrane binding of annexin V	Increased	Increased	Ruf et al (1997); Tomer et al (2001)
Platelet thrombospondin content	N/A	Decreased	Browne et al (1996)
Platelet reduced glutathione levels	Decreased	Decreased	Amer et al (2006)
Urinary thromboxane A2 and prostacyclin metabolites	Increased	Increased	Eldor et al (1991, 1993); Foulon et al (1993)

N/A, not available.

properties to antithrombin, is also decreased in both SCA and β-thalassaemia (Porter *et al*, 1993). HCII levels have been reported to increase following chronic blood transfusions in these patients (O'Driscoll *et al*, 1995). However, it is uncertain whether HCII deficiency is a risk factor for thrombosis (Bertina *et al*, 1987).

Thrombophilic DNA mutations

Patients with SCA appear to be more resistant to activated protein C than the healthy control subjects (Wright *et al*, 1997b). This may result from an increase in circulating plasma levels of factor VIII coagulant activity, perhaps coupled with the reduction in both total and free protein S that is observed in these individuals. However, mutations in the factor V gene (G1691A or factor V Leiden) and the prothrombin gene (i.e. G20210A) is quite low in individuals of African descent. Despite reports that they do not appear to be associated with thrombotic complications in patients with SCD (Kahn *et al*, 1997; Andrade *et al*, 1998), the low frequency of these two alleles makes it difficult to determine their possible contribution to the development of thrombotic complications in these patients.

A study of 50 Lebanese patients with β-thalassaemia intermedia reported a high prevalence for both the factor V Leiden and methylene tetrahydrofolate reductase mutations, and a relatively low prevalence for the prothrombin mutation without significant impact on thrombotic risk (Zalloua *et al*, 2003). In similar studies of patients with β-thalassaemia major, increased prevalence of congenital thrombophilic mutations have not been found (Iolascon *et al*, 2001). From these limited available data, DNA mutations in coagulation factors do not appear to play a significant role in the pathogenesis of thrombosis observed in both SCA and β-thalassaemia.

Endothelial, monocyte and granulocyte activation

Circulating endothelial cells (EC) have been identified in patients with both SCA and β-thalassaemia (Solovey *et al*, 1997, 1998; Butthep *et al*, 2002). The expression of endothelial adhesion molecules and TF on the surface of microvascular activated EC play significant roles in the recruitment of white blood cells and RBC (Carlos & Harlan, 1994; Springer, 1994) and promote thrombosis at sites of vascular inflammation (Mann *et al*, 1998). Red blood cells from patients with β-thalassaemia major and intermedia show enhanced adhesion to cultured EC (Hovav *et al*, 1999). Similar findings are described in SCA (Hoover *et al*, 1979; Hebbel *et al*, 1980), where the interaction of RBC with EC induces a state of oxidative stress with consequent transendothelial migration of monocytes (Sultana *et al*, 1998).

Tissue factor is abnormally expressed on circulating EC in patients with SCA and increases further during pain episodes (Solovey *et al*, 1998). Although, plasma TF antigen and TF-PCA are elevated in patients with SCA in the non-crisis,

steady state, there appears to be no difference in TF-PCA between those patients in steady state and those patients with an acute pain episode (Key *et al*, 1998). TF is a transmembrane protein that provides for calcium-dependent binding of coagulation factor VII and its activated form, VIIa (Nemerson, 1992), and is the principal initiator of coagulation. Therefore, EC expression of TF can play a role in the tightly regulated activation of coagulation. Microparticles (MP), which are small membrane-derived vesicles released by cells following activation or during apoptosis, are TF positive and are derived from RBC, platelets, EC and monocytes (Shet *et al*, 2003). Both MP and TF-positive MP are increased in SCA patients, and even more so during pain episodes. The numbers of MP are significantly higher in patients with β-thalassaemia/Hb E than in normal controls (Pattanapanyasat *et al*, 2004, 2007), but there are no reports on MP in patients with β-thalassaemia major or intermedia.

Multiple plasma factors that could increase TF expression, including thrombin, interleukin-1, tumour necrosis factor and endotoxin, are elevated in patients with SCA, as well as β-thalassaemia. Several potential mechanisms for increased TF expression in SCA include ischaemia-reperfusion injury (Solovey *et al*, 2004), increased levels of the inflammatory mediator, sCD40L (Lee *et al*, 2006), and, possibly, increased haemolysis of type II PS-positive cells (B. N. Y. Setty, 2006, unpublished observations). There is a correlation between the high levels of sCD40L observed in patients with SCD and increased TF antigen, suggesting a contribution of sCD40L to the hypercoagulable state observed in these patients (Lee *et al*, 2006). Furthermore, when lysates of monocytic THP-1 cells were assayed for TF following incubation with plasma from either SCD patients or normal volunteers, the plasma from SCD patients induced a significant increase in TF production relative to plasma from normal individuals or media alone, suggesting a contribution of the CD40–CD40L interaction to the TF expression by monocytes (Lee *et al*, 2006).

Nitric oxide

Nitric oxide (NO) is produced in the endothelium by endothelial NO synthase enzyme, in an oxygen-dependent conversion of arginine to citrulline. It binds to the haem moiety of soluble guanylate cyclase to activate the enzyme, resulting in a conversion of GTP to cGMP, activating cGMP-dependent protein kinases and producing vasodilation (Ignarro *et al*, 1987). Studies in SCA demonstrate plasma Hb-mediated and oxygen free radical-mediated consumption of NO (Gladwin *et al*, 2003; Reiter & Gladwin, 2003), producing a state of NO resistance.

Pulmonary hypertension, a common complication in both β-thalassaemia and SCD (Ataga *et al*, 2004; Gladwin *et al*, 2004; Aessopos & Farmakis, 2005) appears to be caused, at least in part, by chronic intravascular haemolysis with resultant scavenging of NO. In addition to vasodilation, NO has several effects in the vasculature, including anti-adhesive,

antithrombotic and anti-oxidant properties. By inhibiting platelet adhesion and aggregation (Radomski et al, 1987) as well as endotoxin- and cytokine-induced expression of TF and the prothrombotic potential of the EC (Yang & Loscalzo, 2000), NO may play a role in the hypercoagulable state observed in SCA and β-thalassaemia.

Therapeutic implications

The available data on the use of anticoagulants or antiplatelet agents in β-thalassaemia and SCA are either lacking or involve small, poorly controlled and/or relatively low-quality studies. In addition, several treatments in patients with β-thalassaemia and SCA appear to affect the coagulation system in these patients.

Alteration of red blood cells

Red cell transfusion, an important treatment modality in SCA and β-thalassaemia, when given prophylactically significantly reduces the risk of strokes in children with SCA (Adams et al, 1998), a finding that may be related to normalization of the levels of F1.2 (Styles et al, 1997). However, another study found no significant reduction in thrombin generation when children with SCA undergoing chronic transfusion were compared with normal controls (Liesner et al, 1998). The

reduction of thromboembolic complications in adequately transfused patients with β-thalassaemia may be the result of decreased numbers of pathological RBC exhibiting indices of membrane damage (Cappellini et al, 2000).

Treatment with the fetal Hb-inducing agents, hydroxycarbamide and decitabine, result in decreases in plasma markers of thrombin generation (Orringer et al, 1996; Saunthararajah et al, 2003). Hydroxycarbamide, specifically approved for the treatment of SCA, may decrease coagulation activation by reducing PS expression on the surface of both RBC and platelets (Covas et al, 2004) and decreasing RBC adhesion to thrombospondin (Hillery et al, 2000). In addition to being a NO donor (Glover et al, 1999; Gladwin et al, 2002), hydroxycarbamide may also decrease haemostatic activation by its effect in decreasing the white blood cell count (Charache et al, 1996) and particularly monocytes that express TF. While so far, hydroxycarbamide is only rarely used in thalassaemia (Karimi et al, 2005), these patients may experience the benefits because of similar mechanisms described in SCA.

Antiplatelet agents

There are only a few reports on the use of antiplatelet agents in SCA (Haut et al, 1973; Chaplin et al, 1980; Osamo et al, 1981; Greenberg et al, 1983; Cabannes et al, 1984; Zago et al, 1984). (Table II). However, most of these studies failed to

Table II. Published studies of anticoagulants and antiplatelet agents in patients with sickle cell disease.

Reference	Genotype	No. of Subjects	Therapy	Randomized	Duration	Efficacy outcomes and results
Salvaggio et al (1963)	Hb SS	12	Warfarin	No	12–34 months	Modest decrease in frequency of VOEs
Chaplin et al (1989)	Hb SS	4	Heparin	No	2–6 years	Reduced frequency of VOEs
Wolters et al (1995)	Hb SS Hb SC	6 1	Aceno coumarol	No	2 months	Reduced prothrombin fragment 1.2
Schnog et al (2001)	Hb SS Hb SC	14 8	Aceno-coumarol versus placebo	Yes	14 weeks	Reduced markers of coagulation activation, but no reduction of VOE with active treatment
Chaplin et al (1980)	Hb SS	3	ASA/Dipyridamole	No	104 weeks	Modest decrease in frequency of VOE, platelet count and fibrinogen level
Osamo et al (1981)	Hb SS	100	ASA	Yes	6 weeks	Increase in O_2 affinity, Hb and RBC life span
Greenberg et al (1983)	Hb SS Hb SC Hb S-OArab	40 8 1	ASA versus placebo	Yes	21 months	No decrease in frequency of VOE
Semple et al (1984)	Hb SS Hb S-β-Thal	8 1	Ticlopidine versus placebo	Yes	4 weeks	No improvement in frequency of VOE or platelet survival, but decrease in platelet release products
Cabannes et al (1984)	Hb SS	140	Ticlopidine versus placebo	Yes	6 months	Reduction of frequency and duration of VOE
Zago et al (1984)	Hb SS Hb S-β Thal	25 4	ASA versus placebo	Yes	5 months	No differences in frequency of VOE, Hb, reticulocytes, irreversibly sickled cells and fetal haemoglobin level

VOE, vaso-occlusive episode; ASA, aspirin; Hb, haemoglobin; O_2, oxygen; RBC, red blood cell.

correlate the *in vivo* effect of the drugs on platelet activation with specific clinical endpoints. Published studies of anti-platelet agents in patients with β-thalassaemia are limited to two small studies that show improvements in oxygen saturations following the administration of aspirin and dipyridamole to patients with β-thalassaemia/Hb E disease and β-thalassaemia major (Fucharoen *et al*, 1981; Sumiyoshi *et al*, 1992).

Despite these limited and somewhat disappointing results, it remains possible that properly designed studies of antiplatelet agents, administered at doses that will be sufficient to inhibit platelet activation, may have a role in the treatment of certain complications related to SCA and/or β-thalassaemia.

Anticoagulants

Several studies have been carried out in SCA using warfarin (Salvaggio *et al*, 1963), acenocoumarol (Wolters *et al*, 1995; Schnog *et al*, 2001) and mini-dose heparin (Chaplin *et al*, 1989) (Table II). Unfortunately, no meaningful conclusions can be drawn from these studies because of the small number of patients and/or the short-study durations. Although prophylactic antithrombotic therapy has been suggested for patients with β-thalassaemia intermedia at risk for thrombotic complications, such as postsurgery, during pregnancy and immobilization, the results of long-term follow-up studies are currently unavailable. No data are available on the use of thrombolytic agents either in SCA or β-thalassaemia.

Conclusions

On the basis of the available data, the preponderance of evidence demonstrates unequivocally that there is increased platelet and coagulation activation both in SCA and β-thalassaemia. This coagulation activation is probably a consequence of the pathological lipid membrane structure, i.e. the exposure of PS on the outer surface of the RBC. Additional contributing factors include the absence of the spleen and the consequent increase in the number of circulating abnormal RBC and activated platelets, ischaemia-reperfusion injury and, possibly, increased NO scavenging as a result of the increased fragility of type II PS-positive red cells.

Despite the similarities in the pathogenesis of certain complications in both SCA and β-thalassaemia, there remain several important questions. As the pathogenesis of vaso-occlusion in SCA is multifactorial, the exact contribution of hypercoagulability remains to be determined. In addition, several parameters of coagulation and platelet activation have been measured in one disease and not the other. For instance, unlike in SCA, there are no data on the possible role of TF in patients with β-thalassaemia. As a result, further studies are required to extend and confirm the pathogenesis of the observed hypercoagulable state in both diseases.

Defining the contribution of the hypercoagulable state to the pathophysiology of both SCA and β-thalassaemia requires further studies using transgenic animal models to determine the role of the pathological RBC. In addition, with the increasing evidence of the role of NO in the pathophysiology of both SCA and β-thalassaemia, further studies evaluating its contribution to the haemostatic activation observed in these diseases are required. Considering the scientific merits, as well as the therapeutic benefits of studying treatments aimed at reducing hypercoagulability, we suggest the initiation of well-controlled clinical trials of anticoagulants and/or antiplatelet agents employing clinical endpoints that are associated with macrovascular thrombosis (e.g. stroke and pulmonary hypertension). These studies should include the measurement of well-known laboratory markers of platelet and/or coagulation activation.

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