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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Nyffenegger et al, page 769

Isn't it ironic: better RBCs by blocking iron

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In this issue of *Blood*, Nyffenegger et al[1] provide evidence that pharmacologic iron restriction improves disease parameters in the Townes mouse model of sickle cell disease (SCD). Prior studies in this model demonstrated that limiting iron absorption (by intestinal knockout of HIF 2α[2] or by a low-iron diet[3]) led to hematologic improvements. To examine a pragmatic approach to iron restriction, the investigators used vamifeport, an orally administered inhibitor of the cellular iron exporter ferroportin.[4] Ferroportin is the only known means for cellular iron release, and functional loss is embryonic lethal.[5] However, manifestations of haploinsufficiency are seen primarily in cell types with high iron turnover (reticuloendothelial macrophages, enterocytes, and hepatocytes). Patients carrying an inactivating ferroportin allele have a generally mild condition (ferroportin disease type 4A), characterized by hepatic iron loading, relatively low serum iron, and marginally iron-restricted erythropoiesis. The latter consequence has been successfully advantaged in murine β-thalassemia[4] where ferroportin inhibition using vamifeport improved multiple hematologic parameters (see table).

The rationale behind the use of iron restriction in SCD differs from β-thalassemia. In β-thalassemia, the consequent decrease in heme production is thought to attenuate erythroblast oxidative stress and apoptosis, leading to improved erythrocyte production. The decrease in hemichrome formation is thought to improve erythrocyte lifespan. By contrast, in SCD, the intent is to decrease erythrocyte hemoglobin concentrations, based on the observation that even a small decrement in sickle hemoglobin results in a large extension of the time delay between deoxygenation and sickling.[6] As such, iron restriction may provide a way of "buying time" for erythrocytes to escape the hypoxic microvasculature before sickling. Vamifeport appears now to be effective in each of these settings.

As with dietary iron restriction, vamifeport treatment in murine SCD was associated with decreased mean corpuscular hemoglobin concentrations, and a decreased propensity of erythrocytes to sickle (ex vivo by oxygenscan ektacytometry). Although vamifeport treatment did not change the point of sickling (the oxygen tension at which sickling is initiated), it decreased the magnitude of sickling at the lowest oxygen tension (as reflected in the minimum elongation index). These observations support the hypothesized delay time mechanism, and the possibility that iron restriction attenuates subsequent events initiated by microvascular sickling, even if the number of circulating permanently sickled cells remains unchanged. Improvement in hypoxia-induced sickling with vamifeport was associated with decreases in markers of extravascular hemolysis, inflammation, and vascular adhesion. Hemolysis-induced inflammation is a major contributor to the initiation of vaso-occlusive crisis.

Unlike the setting of murine β-thalassemia, vamifeport did not improve anemia in the sickle mice. Indeed, only a modest increase in hemoglobin[2] or hematocrit[3] was reported in prior studies with iron restriction. In both β-thalassemia and SCD mice, vamifeport decreased circulating iron, decreased the mean corpuscular hemoglobin, and decreased the mean corpuscular volume. However, only β-thalassemic mice demonstrated improved erythropoiesis (as evidenced by increased mature marrow erythrocytes) or increased red blood cells. The consequent increase in blood hemoglobin and decrease in erythropoietin plausibly contributed to the observed decrease in splenic size and reticulocyte count. A decrease in splenic size was also observed in the sickle mice with vamifeport treatment, likely from decreased splenic erythrocyte clearance. Interestingly, the corpuscular hemoglobin concentration was decreased in murine SCD by vamifeport but increased in β-thalassemia. This was observed even though mean corpuscular volume was decreased by vamifeport in both conditions. Plausibly, in the sickle mice, there was selective improvement in survival of those erythrocytes with lowest hemoglobin concentrations. Additional studies will be needed to investigate these possibilities.

Despite these differences, there are many overlapping effects of vamifeport in murine SCD and β-thalassemia. Among the most striking consequences in the sickle mice is a decrease in the number of circulating neutrophils. Neutrophil count in SCD is a marker of inflammation and risk for complications.[7] Neutrophil counts were also decreased in β-thalassemic mice with vamifeport. Here also, the neutrophil count may be an indirect marker of hemolysis-triggered inflammation. On the other hand, perhaps the inflammatory response to hemolysis is itself affected by ferroportin inhibition. Neutrophils express ferroportin, and iron status has been shown to affect oxidative burst capacity in other settings.[8] Erythrocytes also express ferroportin[9]; however, the net effect of systemic ferroportin inhibition in the sickle mice is decreased rather than increased iron.

Comparison of changes in iron, hematologic, and erythropoietic parameters in 6- to 7-week-old Townes sickle mice treated with 60 mg/kg vamifeport twice daily for 6 weeks and 8- to 12-week-old β-thalassemia intermedia (th3/+) mice[3] treated with 30 or 100 mg/kg vamifeport twice daily for 36 days

Selected outcomes		Townes sickle mice		Th3/+ β-thalassemia mice	
Parameter	Marker	Compared with control	Response to vamifeport	Compared with control	Response to vamifeport
Iron	Serum	High	↓	Similar	↓
	Liver	High	↔	High	↔
	Liver incorporation	High	↓	High	↓
Hematologic	Mean corpuscular hemoglobin concentration	Low	↓	Low	↑
	Mean corpuscular hemoglobin	High	↓	Low	↓
	Mean corpuscular volume	High	↓	Low	↓
	Red blood cells	Similar	↔	Low (slight)	↑
	Hemoglobin	Low	↔	Low	↑
Erythropoietic	Reticulocytes	High	↔	High	↓
	Splenic index	High	↓	High	↓
	Mature marrow erythrocytes	Low	↔	Low	↑

All mice were fed low-iron chow at the onset of treatment and received iron supplemented water in the 6-hour timeframe between vamifeport doses.

Phase 2 studies have been undertaken examining the effects vamifeport in both β-thalassemia and SCD, although recruitment has recently been put on hold for a reassessment of study design. Phase 1 studies in healthy volunteers suggest vamifeport is well tolerated.[10] Single doses transiently reduced serum iron concentrations, although they rebounded slightly higher. Multiple doses decreased transferrin saturations. Oral availability and a fairly short half-life allow for convenience and easily altered dosing. There may be a number of challenging issues in assessing response to vamifeport in human patients with SCD. The iron status is protean (affected by transfusions), and markers of iron status can be difficult to interpret (affected by inflammation). Hepcidin status is variable as well, with its own effects on ferroportin-mediated iron export. Finally, the therapeutic window might be narrow, with potential risk for tissue iron deficiency if excessive. As an outcome target, markers of hemolysis or inflammation (including neutrophil count) may prove more useful than hemoglobin levels. Oxygenscan ektacytometry may allow for convenient measures of propensity to sickle. The most important outcome will be a decrease in SCD-related complications.

The current study provides exciting proof-of-concept for pharmacologic manipulation of iron status as an additional therapeutic approach for patients with SCD and identifies some interesting avenues for future investigation. We look forward to results of initial clinical investigations.

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