Potential Utility of Full-Spectrum Antioxidant Therapy, Citrulline, and Dietary Nitrate in the Management of Sickle Cell Disease

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Abstract

There is considerable evidence that oxidative stress and a loss of nitric oxide bioactivity are key mediators of the vasculopathies associated with sickle cell disease. A comprehensive nutraceutical strategy for mitigating the contribution of oxidative stress to pathogenesis – dubbed “full-spectrum antioxidant therapy” – may have utility in this syndrome. This strategy entails concurrent administration of phycocyanobilin – a phytochemical richly supplied by spirulina, shown to inhibit NADPH oxidase in a manner analogous to its chemical relatives biliverdin and bilirubin; high-dose folate – recently shown to quench peroxynitrite-derived radicals and restore coupling of NO synthase; N-acetylcysteine – for boosting intracellular glutathione levels; and a phase 2 inducer such as lipoic acid – to further promote glutathione synthesis while increasing expression of antioxidant enzymes. Suboptimal endothelial arginine levels, reflecting increased plasma arginase activity and elevated ADMA, contribute to the loss of NO bioactivity in sickle cell disease; supplementation with the arginine precursor citrulline may ameliorate this defect. Increased intakes of plant-derived nitrate have the potential to diminish the quenching of NO by plasma hemoglobin in sickle cell patients, while boosting systemic NO production independent of NO synthase activity. In addition to the well-documented utility of hydroxyurea – possibly a suboptimal strategy for life-long therapy owing to its mutagenic activity- rational pharmaceutical options for managing sickle cell disease include pentoxifylline and phosphodiesterase 5 inhibitors such as sildenafil.

Role of Oxidative Stress and Loss of Nitric Oxide Bioactivity in Sickle Cell Syndrome

There is considerable evidence and speculation that oxidative stress and impaired nitric oxide (NO) function are key mediators of the chief vascular complications of sickle cell disease.1,2 Oxidative stress is induced in tissues subjected to ischemia-reperfusion consequent to intermittent vasoocclusion triggered by dense sickled erythrocytes. It also arises in erythrocytes owing to polymerization of hemoglobin S (HbS), promoting the irreversible sickling and dense cell formation that lead to vaso-occlusive episodes, and also likely promoting intravascular hemolysis. Oxidative stress, in turn, impedes the synthesis and bioactivity of nitric oxide by uncoupling nitric oxide synthase (thereby further increasing oxidative stress) and by superoxide-mediated quenching of NO, which gives rise to dangerous peroxynitrite. NO is also quenched by by interaction with free hemoglobin in the plasma and extracellular space, stemming from intravascular hemolysis of sickled erythrocytes; this hemoglobin mediates a reduction of NO, converting it to inactive nitrate. Loss of NO bioactivity and oxidative stress are mediators of tissue damage and dysfunction in tissues affected by vaso-occlusion, but they also increase risk for further vaso-occlusion by pro-inflammatory effects that render endothelial cells more adhesive and chemoattractive for circulating leukocytes, and that promote thrombotic mechanisms. Hence, vicious cycles develop in which oxidative stress, loss of NO activity, and vaso-occlusion become mutually reinforcing. Typical pathological manifestations of these
effects include pulmonary hypertension, stroke, priapism, splenic infarction, and avascular necrosis.

**Controlling Oxidative Stress in Sickle Cell Patients – Full-Spectrum Antioxidant Therapy**

Nutraceutical strategies for quelling oxidative stress and restoring effective NO activity may have considerable potential for the management of sickle cell disease. Full-spectrum antioxidant therapy (FSAT) has been defined as a supplementation program that incorporates phycocyanobilin (the chief phytonutrient of spirulina), high-dose folic acid, a clinically effective phase 2 inducer such as lipoic acid, and N-acetylcysteine; its intent is to minimize the contribution of oxidative stress to the pathogenesis of a wide range of disorders. Phycocyanobilin (PhyCB), a biliverdin derivative, has recently been shown to mimic the inhibitory impact of biliverdin/bilirubin on NADPH oxidase activity. Activated NADPH oxidase seems likely to be a key source of pro-inflammatory oxidative stress in endothelia affected by episodic vaso-occlusion; in particular, in the cerebral microvasculature of transgenic betaS mice (a murine model for sickle cell disease), this enzyme complex has been shown to be a prime mediator of the increased endothelial adhesiveness for leukocytes and platelets triggered by transient hypoxia. Moreover, pre-administration of biliverdin to transgenic sickle mice prevents the increase in subcutaneous venular stasis provoked by one hour of hypoxia followed by reoxygenation. In rodents, oral administration of whole spirulina or of phycocyanin, the holoprotein which contains PhyCB as a covalently attached chromophore, has shown a diverse range of anti-inflammatory and cytoprotective effects that seem likely to reflect, at least in large measure, PhyCB-mediated inhibition of NAPDH oxidase activity. Thus, oral PhyCB may have potential for controlling a portion of the pro-inflammatory oxidative stress associated with sickle cell disease.

The recently appreciated antioxidant potential of high-dose folate reflects the fact that many tissues (including vascular endothelium) actively concentrate this vitamin and rapidly convert it to reduced forms – tetrahydrofolates – which have diverse and potent scavenging antioxidant activity. In particular, tetrahydrofolates efficiently scavenge peroxynitrite-derived oxidants – an effect which likely explains the ability of high-dose folate administration to protect endothelial tetrahydrobipterin from oxidative damage, effectively recoupling NO synthase activity in oxidant-stressed endothelium. Uncoupling of NO synthase is believed to play a key role in the loss of NO biosynthetic capacity and the increased endothelial oxidative stress observed in tissues affected by sickle cell disease; thus, high-dose folate may have potential for reversing these effects. Moreover, pre-administration of high-dose folate has recently been shown to aid preservation of myocardial function and prevent cardiomyocyte death in rats subjected to transient left coronary artery occlusion; scavenging of peroxynitrite-derived radicals likely plays a role in this effect, but prevention of mitochondrial oxidative damage evoked by hypoxia might also be involved. Thus, high-dose folate may have potential for preserving NO bioactivity and blunting oxidative damage during and following vaso-occlusive episodes, and merits evaluation in transgenic sickle mice.

Glutathione is the major intracellular scavenging antioxidant, and, in its reduced form, acts as a functional antagonist of the pro-inflammatory signaling evoked by hydrogen peroxide. Glutathione synthesis can be promoted by administration of N-acetylcysteine (NAC), which
boosts intracellular levels of cysteine, the rate-limiting substrate for glutathione production, and by phase 2 inducer chemicals that increase expression of gamma-glutamylcysteine synthase, the enzyme that is rate-limiting for glutathione synthesis.\textsuperscript{24-27} Both in vitro and in vivo, NAC has shown the capacity to boost glutathione levels in HbS erythrocytes – an effect which tends to prevent irreversible sickling and dense cell formation.\textsuperscript{28, 29} In a phase II double-blind study, daily oral NAC was found to inhibit dense cell formation and decrease the frequency of vaso-occlusive episodes at the optimal dose of 2400 mg daily, the rate of these episodes was decreased by about 60% relative to placebo-treated patients.\textsuperscript{29} The possibility that NAC might also have a favorable effect on the endothelial inflammation associated with sickle cell disease should also be considered.

Phase 2 inducers may also have potential in the management of sickle cell disease – not only by promoting glutathione synthesis, but by increasing the expression a certain antioxidant enzymes.\textsuperscript{26, 30} In particular, they may boost expression of heme oxygenase-1, which catabolizes the pro-oxidant heme, in the process generating the antioxidant biliverdin and cytoprotective carbon monoxide. In transgenic sickle mice, the HO-1 inducer hemin has been shown to counteract hypoxia-evoked venular stasis.\textsuperscript{10} Lipoic acid is a phase 2 inducer that has well documented clinical utility in the management of diabetic neuropathy, and that has been shown to boost glutathione levels in diverse tissues in rodents.\textsuperscript{31-34} In rodent studies, it has shown protective antioxidant effects in models of ischemia-reperfusion damage and in circumstances that subject erythrocytes to oxidative stress.\textsuperscript{35-42} Although lipoic acid supplementation did not notably influence markers of oxidative stress in a recent controlled trial in sickle cell subjects, the daily dose chosen – 200 mg – was far lower than that shown to be useful in diabetic neuropathy, and thus may have been inadequate.\textsuperscript{43} Thus, lipoic acid merits further evaluation in this regard.

**Citrulline for Arginine Deficiency**

It is suspected that induced arginine deficiency – stemming from intravascular cleavage of arginine by the arginase released from lysed erythrocytes – contributes to the loss of NO bioactivity associated with sickle cell disease.\textsuperscript{2, 44} Indeed, plasma levels of arginine are reported to be subnormal in adults, though not children, with sickle cell disease; they decrease in both children and adults during vaso-occlusive crises.\textsuperscript{45} Good arginine availability may be of especial importance to efficient NO synthase activity in sickle cell patients in light of the fact that endothelial levels of asymmetric dimethyl arginine (ADMA), a competitive antagonist of arginine in the NO synthase reaction, are elevated in these patients.\textsuperscript{46} Indeed, arginine supplementation typically enhances NO synthesis in the context of elevated ADMA levels.\textsuperscript{47, 48} However, supplementation with citrulline, rather than arginine per se, may be a more efficient way of increasing plasma and tissue levels of arginine, and such supplementation may be particularly appropriate in sickle cell patients owing to their elevated plasma levels of free arginase.\textsuperscript{49-51} The superiority of citrulline as a delivery form for arginine reflects the fact that supplementary arginine is largely degraded by arginase activity in the GI tract and intestinal mucosa before it can reach the circulation. Orally administered citrulline escapes this degradation, and, once absorbed, is gradually converted by the kidneys to arginine, thereby boosting plasma arginine levels. However, such supplementation also produces increases in plasma citrulline,\textsuperscript{49} which has the potential to be taken up by endothelial cells and employed for
NO synthesis. Indeed, there is evidence that a high proportion of the citrulline produced in endothelial cells as a by-product of NO synthase activity is rapidly reconverted to arginine by enzymes (argininosuccinate synthase, argininosuccinate lyase) found in close proximity to NO synthase in these cells.\textsuperscript{52, 53} Thus, by bypassing arginase activities not only in the GI tract and intestinal mucosa, but also in plasma, citrulline supplementation may be particularly appropriate as a strategy for assuring intracellular arginine levels that support optimal NO synthase activity. Indeed, in an open phase 2 clinical trial of supplemental citrulline (0.1 g/kg/day) in sickle cell patients, a markedly favorable impact on symptoms was observed.\textsuperscript{50} Controlled studies are evidently needed to confirm this intriguing preliminary observation.

Arguably, measures intended to boost NO synthase activity by optimizing arginine availability should be used in conjunction with antioxidant measures that decrease superoxide levels or that mitigate the oxidant damage induced by peroxynitrite; otherwise, increased NO production will be accompanied by increased production of peroxynitrite and increased risk of peroxynitrite-mediated adverse effects. Thus, citrulline supplementation may be most worthwhile when accompanied by antioxidant measures such as those suggested above.

**Dietary Nitrate May Ameliorate the Adverse Impact of Plasma Hemoglobin on NO**

As noted above, loss of NO bioactivity in sickle cell disease and other hemolytic disorders reflects, in part, reductive quenching of NO by deoxyhemoglobin in the plasma and extracellular space.\textsuperscript{1} Theoretically, this effect could be offset by administration of an innocuous molecule capable of oxidizing plasma hemoglobin. Indeed, parenteral administration of sodium nitrite has been used for this purpose, and it has been suggested that this strategy might be useful for managing acute sickle cell crises – in part because reduction of nitrite generates NO.\textsuperscript{54} Although excessive nitrite exposure would be counterproductive owing to induction of methemoglobinemia, a sufficiently low concentration of nitrite might be expected to influence plasma hemoglobin selectively, not only because erythrocyte membranes act as a diffusion barrier, but also because erythrocytes have mechanisms for restoring the proper reduced state of hemoglobin.

It is intriguing to note that diets rich in green vegetables can provide over a gram of nitrate per day – and much of this nitrate, after absorption, is secreted in saliva, where oral bacteria reduce it to nitrite.\textsuperscript{55-57} Thus, oral consumption of nitrate boosts plasma nitrite levels. Could high-normal dietary levels of nitrate – or perhaps slightly higher levels – have a favorable impact on hemoglobin-mediated NO quenching in sickle cell patients, without provoking an unacceptable level of methemoglobinemia? Or could some other small molecule capable of oxidizing plasma hemoglobin be useful in this regard? These possibilities merit consideration. Intriguingly, ingestion of 500 ml of beetroot juice, containing 1.4 g nitrate, was found to cause a delayed but substantial reduction in the blood pressure of healthy volunteers; the maximal hypotensive effect coincided with the induced elevation of plasma nitrite.\textsuperscript{57} It would be interesting to determine whether such a regimen could achieve a worthwhile conversion of plasma hemoglobin to methemoglobin in sickle cell patients; in any case, the increased NO evolved by this strategy – not dependent on arginine availability or NO synthase activity – would likely be beneficial to these patients.
A recent analysis of nitrate contents in common vegetables noted high nitrate contents - >250 mg/100 g wet weight – in celery, cress, chervil, lettuce, red beetroot, spinach, and ru cola.\(^5\) Spinach was highest, at 741 mg/100 g; hence, a diet rich in spinach may have potential in the management of sickle cell disease.

**Pharmaceutical Options**

The only drug currently approved for treatment of sickle cell disease is the cytotoxic agent hydroxyurea, which increases the expression of the fetal isoform of hemoglobin in erythrocytes, thereby reducing their tendency to sickle.\(^5\) However, lifelong use of this agent may well entail increased risk for mutagenesis and cancer. An alternative pharmaceutical strategy for decreasing risk for vaso-occlusive episodes – a strategy which so far has received inadequate clinical attention – is administration of pentoxifylline. Several clinical groups have evaluated pentoxifylline in sickle cell patients – either in an effort to abort ongoing crises, or to prevent them – and most of these have concluded that pentoxifylline is useful in this regard.\(^6\) This is not terribly surprising, as pentoxifylline’s well documented utility in peripheral artery disease reflects its ability to reduce blood viscosity and render erythrocyte membranes more distensible.\(^6\) Given the fact that pentoxifylline is a safe, well tolerated, and relatively affordable drug with decades of prior use, a more substantial clinical effort to evaluate its utility in sickle cell disease would appear to be warranted.

Another category of available drugs likely to be useful in sickle cell disease are the phosphodiesterase 5 inhibitors approved for use in erectile dysfunction. These potentiate the aspects of NO bioactivity mediated by cGMP, as they increase the half-life of this mediator. Pilot clinical studies with sildenafil in sickle cell patients with pulmonary hypertension have demonstrated a worthwhile reduction in pulmonary blood pressure accompanied by a significant increase in walking distance.\(^6\) Since priapism is a common chronic complication of sickle cell disease, it is reassuring that this therapy did not trigger priapism. A potential herbal alternative to these still expensive drugs may be offered by Epimedium sagittatum (a.k.a. horny goat weed!), which is rich in a compound, icariin, shown to inhibit phosphodiesterase 5 in submicromolar concentrations.\(^6\)-\(^7\) Anecdotally, extracts of this herb standardized for 10% icariin content are claimed to be useful in erectile dysfunction, although the onset of action is said to require several days; there are currently no published controlled studies confirming these claims.

**References**


(4) McCarty MF. The Oasis Executive Health Program. 2009. Ref Type: Unpublished Work


(10) Belcher JD, Mahaseth H, Welch TE, Otterbein LE, Hebbel RP, Vercellotti GM. Heme oxygenase-1 is a modulator of inflammation and vaso-occlusion in transgenic sickle mice. *J Clin Invest* 2006 March;116(3):808-16.


(52) Goodwin BL, Solomonson LP, Eichler DC. Argininosuccinate synthase expression is required to maintain nitric oxide production and cell viability in aortic endothelial cells. *J Biol Chem* 2004 April 30;279(18):18353-60.

(53) Flam BR, Eichler DC, Solomonson LP. Endothelial nitric oxide production is tightly coupled to the citrulline-NO cycle. *Nitric Oxide* 2007 November;17(3-4):115-21.


