Rescuing Decrepit Soluble Guanylate Cyclase: A Therapy for Sickle Cell Disease?

Sickle cell disease (SCD) is a severe hemoglobinopathy that arises from a point mutation in the gene encoding the hemoglobin (Hb) β chain. In homozygous individuals carrying mutations in both alleles, an abnormal sickle Hb (HbS) is expressed that tends to form a polymeric gel consisting of rigid fibers that distort the shape and flexibility of the erythrocytes. Those changes predispose the cells to rupture and cause damage to microvessels, resulting in a vasculopathy that contributes importantly to pathogenesis of the disease. Individuals with SCD develop chronic anemia and a tendency to experience acute vaso-occlusive crises associated with severe pain. Long-term complications include increased risks of stroke and acute chest syndrome (1). Some individuals with SCD develop pulmonary hypertension (2), for which few effective therapeutic options exist.

The flagship treatment for SCD involves administration of hydroxyurea, a cytotoxic and cytostatic drug that lessens the frequency of vaso-occlusive episodes and other complications, and decreases the length of hospital stays, and does so at low cost and with a fairly favorable safety record (3). However, the effectiveness of hydroxyurea varies considerably among individuals. The mechanisms underlying its beneficial effects are not fully understood but likely relate to the tendency of hydroxyurea to stimulate fetal Hb expression, which lessens the tendency of HbS to undergo polymerization. In addition, hydroxyurea may act by augmenting the generation of nitric oxide (NO), which promotes vasodilation and inhibits platelet aggregation by activating soluble guanylate cyclase (sGC)-mediated production of cyclic guanosine monophosphate. Given the importance of thrombosis and vascular damage in SCD, it would seem that augmenting NO signaling by other means could also be useful in the treatment of SCD. One approach would be to augment the half-life of cyclic guanosine monophosphate using the phosphodiesterase-5 inhibitor sildenafil. However, sildenafil treatment was found to cause a paradoxical increase in acute pain events, making it unsuitable for the treatment of SCD (4).

Other attempts to increase NO signaling face special challenges in patients with SCD. As a consequence of erythrocyte hemolysis, extracellular Hb is chronically increased. Free Hb that migrates into the vascular wall can potentially scavenge NO en route from the endothelium to smooth muscle. Moreover, free Hb in plasma can inhibit the antithrombotic effects of endothelial NO synthase (eNOS)-derived NO on platelets. Another mechanism that undermines NO signaling in SCD can arise from excessive generation of reactive oxygen species (ROS). Although a full understanding of the sources and targets of these oxidants is lacking, possible sources include nicotinamide adenine dinucleotide phosphate oxidases (expressed in inflammatory and other cell types), and uncoupled eNOS. HbS may itself augment ROS generation through its tendency to undergo autooxidation (5). One study reported that extracellular xanthine oxidase is released from the liver during SCD-induced ischemic injury (6). Circulating xanthine oxidase then binds to the vascular wall and, through the generation of superoxide, contributes to the vasculopathy of SCD. Within the vessel wall, ROS (and superoxide in particular) can directly scavenge NO and inactivate eNOS, thereby promoting vasoconstriction in both systemic and pulmonary vascular beds. Upregulation of Hb α in endothelial cells can also inhibit NO signaling (7). An important vascular target of oxidant stress is the ferrous heme iron in sGC, which is oxidized by superoxide. In that decrepit state, sGC is insensitive to NO, as well as to existing drugs (such as riociguat) that stimulate the activity of normal sGC. Thus, multiple mechanisms contribute importantly to a suppression of NO generation, a shortening of its biological half-life, and an inactivation of its downstream targets in SCD, resulting in a chronic state of NO resistance (8).

A significant fraction of individuals with SCD develop pulmonary hypertension, an exacerbation that increases the morbidity and mortality of the disease. Factors that contribute to the onset of this condition likely include erythrocyte hemolysis, pulmonary endothelial damage, and vascular injury resulting from chronic damage by thromboembolic events. The degree of pulmonary arterial hypertension is exacerbated by the relative loss of endogenous NO responsiveness, and treatment options are limited by the associated resistance to therapies aimed at augmenting NO-mediated signaling. How can this problem be solved? In this issue of the *Journal*, Potoka and colleagues (pp. 636–647) describe an alternative strategy involving the use of two novel agents to augment sGC activity (9). One agent acts by stimulating normal (nonoxidized) sGC whether NO is present or not, whereas the other *activates* sGC even if the heme Fe^{2+} has been oxidized or lost altogether. These agents were compared in a genetic mouse model of SCD in which the wild-type mouse Hb genes have been replaced with human sequences that include the sickle cell transgene. These mice—referred to as the Berkeley SCD model—exhibit the sickling behavior seen in the human disease, along with the major histopathologic characteristics of human SCD, including chronic anemia and multiorgan pathology (10). In this model, the robust recapitulation of features seen in human SCD provides an ideal system for studying the potential therapeutic effects of novel agents. Importantly, these mice also exhibit NO resistance, increased ROS generation, congestion of pulmonary vessels, increased pulmonary vascular pressures, and right ventricular hypertrophy.

The sGC stimulator and activator agents were added to the mouse chow, permitting a long-term comparison of their effects in this model. During a 30-day study initiated at 6 months of age, neither agent affected blood pressure, heart rate, or the development of hemolytic anemia. However, measurements of right ventricular systolic pressure (RVSP) revealed that the activator compound, but not the stimulator, decreased the maximal RVSP compared with controls. However, the activator did not attenuate cardiac remodeling, whereas the stimulator caused an unexpected increase

in RV hypertrophic remodeling (Fulton's index), as did sildenafil treatment. The SCD mice also exhibited left ventricular (LV) hypertrophic remodeling, although none of the treatments mitigated that response. Complementary ex vivo studies of vascular reactivity in pulmonary artery segments confirmed the presence of endothelial dysfunction and NO resistance, which were ameliorated in vessels from mice treated for 30 days with the activator compound. These studies underscore the concept that restoration of NO bioavailability is unlikely to prove beneficial in SCD if the target (sGC) is broken. On the other hand, an agent that could restore sGC activity despite its loss of NO sensitivity could be highly effective.

The effects of more prolonged administration were assessed in a 90-day study initiated when the mice reached 4 months of age. Again, maximal RVSP was attenuated by the activator, as was RV remodeling when assessed by RV weight normalized to tibia length. However, there was no significant decrease in Fulton's index (RV/[LV + septum]), possibly because the LV hypertrophy decreased to a greater extent than the RV hypertrophy. NO resistance, assessed in ex vivo studies of excised vessel segments, was not ameliorated by treatment. Collectively, these studies identify the potential beneficial effects on pulmonary hypertension and cardiac remodeling of long-term administration of an sGC activator that works even when the enzyme has been degraded by oxidant stress.

Among the many strengths of this study, the use of a genetic mouse model of SCD that so accurately recapitulates the human disease phenotype stands out. In addition, both short- and long-term administration strategies reveal that the beneficial effects may continue to accrue over time. Analyses of the independent effects of treatment on the right and left ventricles, as well as the inclusion of sildenafil-treated and isogenic control groups, are additional strengths that underscore the validity of their findings.

As with all insightful papers, the results of this study raise some additional questions that future experiments could address. One open question relates to which tissues or cells are responsible for the beneficial effects of the activator, given that the drug was administered systemically and that many cells express sGC. For example, administration of the activator was associated with a decrease in LV hypertrophy, yet the drug had no effect on systemic blood pressure or heart rate—two factors known to affect LV hypertrophic remodeling. This might reflect a direct effect of sGC activation in cardiomyocytes that leads to a suppression of cellular hypertrophic remodeling. Another important target of NO signaling is the platelet, raising the question of whether the drug-mediated rescue of sGC signaling in platelets, which become activated by ongoing intravascular hemolysis in SCD, may have altered coagulation-mediated vascular injury. Pulmonary vascular remodeling is the canonical cause of pulmonary arterial hypertension, so it is curious that treatment with the activator lessened RVSP significantly without producing a comparable salutary effect on vascular wall thickening. Perhaps this indicates that a primary beneficial effect of this activator arises from its ability to inhibit active vascular tone, rather than its ability to modulate inflammatory cell function and vascular cell remodeling. Again, these questions underscore the need to understand which cells are being affected by the restoration of sGC activity in the context of SCD. Finally, as the vasculopathy of SCD affects many organs, it will be interesting to see whether the beneficial effects of this approach extend to other organ systems affected by this disease. ■

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Paul T. Schumacker, Ph.D.
Feinberg School of Medicine
Northwestern University
Chicago, Illinois

ORCID ID: 0000-0001-9591-2034 (P.T.S.).

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