# Comment on "The influence of hydroxyurea on oxidative stress in sickle cell anemia"

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Sickle cell disease (SCD) is a monogenetic disorder caused by single amino acid change in the beta globin gene. This mutation facilitates the production of unstable sickle hemoglobin (Hb S) which upon deoxygenation forms higher order aggregates which cause erythrocyte rigidity. These rigid cells block the microvasculature resulting in vaso-occlusion, tissue ischemia, organ damage, pain and death. The rigid cells tend to lyse easily, releasing hemoglobin, a protein with oxidant properties related to its heme-bound iron. Therefore, the pathophysiology of SCD is driven by the molecular properties of Hb S, the amount of vaso-occlusion, and the rate of hemolysis. All of these three features are implicated in the induction of oxidative stress in SCD: The unstable Hb S has a high rate of spontaneous auto-oxidation inducing superoxide[1]. Intravascular hemolysis results in high levels of free hemoglobin and free heme that have the potential to facilitate hydroxyl-radical formation[2].Vaso-occlusion results in repeated ischemia-reperfusion cycles that likely strongly induce tissue oxidative stress throughout the body. High levels of xanthine oxidase and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activities produce oxygen radicals as part of their normal activities[3]. It also is possible that arginase-catalyzed depletion of the arginine substrate for nitric oxide synthase induces its uncoupling with consequent production of reactive oxygen species[4]. Markers of oxidative stress such as advanced glycation end products are related to organ damage in SCD[5]. Taken together there is no doubt that oxidative stress is prominent in the pathophysiology of SCD.

Currently, hydroxyurea (HU) is the only disease-modifying therapy approved for SCD. Administration of HU increases the synthesis of fetal Hb (Hb F), which inhibits the polymerization of Hb S. In sickle cell mice, augmented Hb F expression reduces oxidant stress[6]. In SCD patients higher Hb F reduces the number of painful vaso-occlusive crises and appears to reduce hemolysis[7,8]. By reducing vaso-occlusion and hemolysis alone, administration of HU could have a major impact on total oxidative stress in SCD. However, there might also be direct effects of HU on the balance between oxidative stress and anti-oxidant capacity. For instance, it has been shown that HU induces glutathione peroxidase, an important anti-oxidant in sickle erythrocytes[9]. Glutathione peroxidase activity plays a role in reducing membrane lipid peroxidation, promoting membrane stability and thereby likely reducing hemolysis.

HU has both direct oxidant and indirect antioxidant properties. Its azide moiety can oxidize hemoglobin to methemoglobin and nitrosyl hemoglobin[10]. Sickle erythrocytes even tend to be more sensitive to this effect of HU[11]. In SCD, however, the production of methemoglobin might not be problematic. Although methemoglobin cannot carry oxygen, it inhibits Hb S polymerization with a potential benefit to reduce hemolysis and vaso-occlusion[12]. HU can be metabolized to nitric oxide, which has antioxidant properties of its own[13].

These complex effects of HU create a difficulty in predicting the net effect of total oxidant stress with the administration of this medication in SCD patients. In this edition of the *Revista Brasileira de Hematologia e Hemoterapia*, Torres et al. evaluate plasma markers of oxidative stress between SCD patients on and off HU, a topic that has been only lightly investigated previously[14]. In a previous study, the same group showed that comparing SCD patients on chronic transfusion and chelation therapy to SCD patients on HU and chelation therapy, the latter had lower levels of markers of lipid peroxidation[15]. Another study found no difference in the expression of genes related to oxidative stress in peripheral blood mononuclear cells of SCD patients on and off HU[16].

In the current study, Torres et al. show a clear difference in the amount of thiobarbituric acid reactive species (TBARS), a commonly applied assay to quantify lipid peroxidation in the plasma of SCD patients on and off HU. They show that SCD patients that are using HU have less lipid peroxidation than SCD patients that do not take HU. They also show a negative correlation in the Hb F level with lipid peroxidation, a finding that parallels previously published results in SCD mice[6].The authors apply the trolox equivalent antioxidant capacity (TEAC) to measure the antioxidant capacity of plasma. This assay gives a global impression of the antioxidant capacity

Conflict-of-interest disclosure:
The authors declare no competing financial interest

Submitted: 11/16/2012
Accepted: 11/20/2012

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DOI: 10.5581/1516-8484.20120099

of plasma but is influenced for instance by bilirubin levels and uric acid[17]. Torres et al. find that SCD plasma has a higher TEAC than control plasma and this TEAC is even higher in SCD patients using HU, suggesting that plasma antioxidant capacity rises in SCD as an adaptation to chronic oxidative stress and that HU further augments antioxidant capacity.

It is even more difficult to interpret the increased plasma levels of glutathione in patients with SCD compared to controls. Glutathione levels in plasma tend to be about 100-fold lower than intracellular erythrocyte levels and are lower in SCD erythrocytes compared to normal erythrocytes[18,19]. Recently, sickle erythrocytes have been shown to export oxidized glutathione at a higher rate than normal erythrocytes[20]. Some of this extracellular oxidized glutathione might be reduced back to glutathione by glutathione reductase in the plasma, some might be released from lysed sickle erythrocytes, and thus might more reflect the activity of this enzyme rather than total body glutathione levels. These complexities make these differences in plasma glutathione difficult to interpret.

Although the results of Torres et al. might be influenced by selection bias of different SCD patients in the HU group (referred to as confounding by indication), results from research by them and others supports the idea that HU attenuates oxidative stress in SCD patients. The strong correlation between Hb F and markers of lipid peroxidation suggest that the anti-oxidant effects of HU are largely attributable to increased Hb F. Whether patients without an increase in Hb F upon administration will benefit from the possible other anti-oxidant effects of HU remains a question.

## Acknowledgements

The authors receive research support from the National Heart, Lung and Blood Institute Division of Intramural Research (1 ZIA HL006014-03 and others). Dr. van Beers is a recipient of research fellowship funding from Novartis. The authors report no conflict of interests in this article.

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