Therapeutic Strategies to Alter the Oxygen Affinity of Sickle Hemoglobin

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KEYWORDS

• Sickle cell • 5-HMF • Antisickling • R state • Hemoglobin allosteric effectors

KEY POINTS

- The T state of sickle hemoglobin (HbS) is prone to polymerize, promoting red cell sickling.
- Stabilizers of the R state of HbS have the potential to directly inhibit sickling.
- R-state stabilizers also increase the affinity of HbS for oxygen.
- An R-state stabilizer, 5-hydoxymethyl-2-furfural (also known as Aes-103) is currently in clinical trials.

OXYGEN AFFINITY OF SICKLE ERYTHROCYTES

The erythrocytes in sickle cell disease have long been known to show decreased oxygen affinity compared with those from healthy volunteers.[1–4] This property is measured as an increase in the partial pressure of oxygen required to produce 50% oxygen saturation (P_{50}), discussed in further detail later. This decreased oxygen affinity is caused at least in part by increased intracellular concentration of 2,3-diphosphoglycerate (2,3-DPG) in erythrocytes, observed generally in all forms of anemia and considered a compensatory adaption that facilitates oxygen release from red cells to the tissues. 2,3-DPG is a product of anaerobic glycolysis, which has been found in recent years to be regulated in erythrocytes by oxygen-regulated sequestration and inactivation of glycolytic enzymes by the cytoskeletal protein band 3.[5–7] Among patients with sickle cell disease, P_{50} and 2,3-DPG levels vary widely, and more increased levels seem to decrease solubility of sickle hemoglobin (HbS),[8–10] and to

Disclosure: See last page of article.
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Hematol Oncol Clin N Am 28 (2014) 217–231
http://dx.doi.org/10.1016/j.hoc.2013.11.001 hemonc.theclinics.com
0889-8588/14/$ – see front matter

increase red cell sickling under hypoxia,[7,11] although this has not been confirmed by all investigators.[12,13] In vitro manipulation of human sickle blood to reduce 2,3-DPG content in red cells also reduces hypoxia-induced sickling in vitro.[14] Preliminary investigation suggests that decreased oxygen affinity of HbS may be associated with greater clinical symptoms,[8,15] but more investigation is needed to confirm this association. Although decreased oxygen affinity may be adaptive in other anemias, it may be counteradaptive in sickle cell disease because of its promotion of the T state of HbS, which promotes sickling. These effects relate to alterations in the conformation of hemoglobin (Hb).

THE ALLOSTERIC STATES OF HB AND SICKLE CELL DISEASE

Hb has been shown to function in equilibrium between 2 classic states: the tense (T) state, which has low affinity for ligand, and the relaxed (R) state, which has high affinity for ligand.[16–19] The crystal structure of the T-state (unliganded or deoxygenated) or the R-state (liganded or oxygenated) Hb is each made up of 2 alpha-beta heterodimers ($\alpha1\beta1$ and $\alpha2\beta2$) arranged around a 2-fold axis of symmetry to form a central water cavity with the alpha cleft and beta cleft defining entries into the cavity (**Fig. 1**). The T→R allosteric transition is characterized by rotation of the $\alpha1\beta1$ dimer relative to the $\alpha2\beta2$ dimer, which significantly reshapes the central water cavity, resulting in several differences between the quaternary T and R structures. Most notable is the formation of a larger central water cavity; including the alpha and beta clefts in the quaternary T structure with respect to the quaternary R structure, as well as several different interdimer ($\alpha1\beta2$ or $\alpha2\beta1$, $\alpha1\alpha2$ and $\beta1\beta2$) hydrogen bond and/or salt-bridge interactions in the T or R structures that stabilize one state relative to the other. Despite the presence of βVal6 in HbS, normal and HbS molecules have identical quaternary structures.

The T and R structures were used to formulate the Monod-Wyman-Changeux[20] and the Koshland-Némethy-Filmer[21] allosteric models and later modified by Perutz[16–19] with his stereochemical construct. Since then, several R-like or T-like conformations within quaternary T and R states,[22–28] as well as distinct quaternary relaxed states (R2, R3, RR2, RR3, and so forth) that extend beyond the classic T→R transition[29,30] have been described and/or incorporated in modern allosteric models.[31,32] Like the

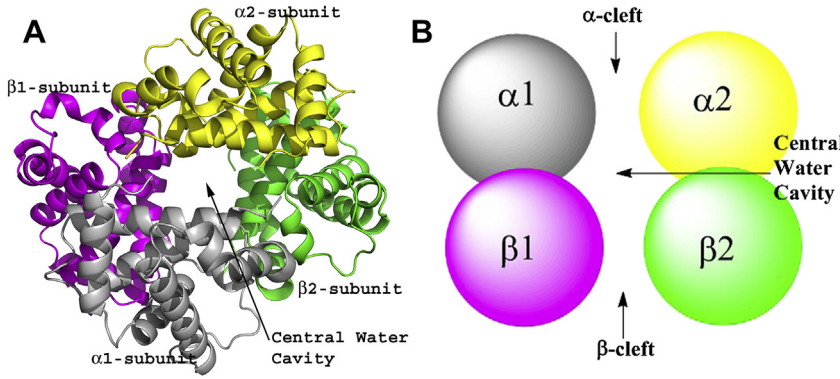

Fig. 1. Quaternary structure of human Hb. Alpha-1 subunit (*gray*), alpha-2 subunit (*yellow*), beta-1 subunit (*magenta*), and beta-2 subunit (*green*). (*A*) Ribbon diagram showing the 4 Hb subunits arranged around a central water cavity. (*B*) The Hb subunits showing access to the central water cavity via the alpha cleft and beta cleft.