GTx011* (GBT440), an Anti-Sickling Compound, Improves SS Blood Rheology by Reduction of HbS Polymerization via Allosteric Modulation of O2 Affinity

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Introduction

Sickle cell disease (SCD) is an inherited disorder caused by a point mutation in the β-globin gene leading to formation of hemoglobin S (HbS). A primary and obligatory event in the molecular pathogenesis of SCD is the polymerization of deoxygenated HbS and the resultant sickling of red blood cells (RBC). Sickle cell disease is characterized by hermetic vaso-occlusions leading to progressive end-organ damage with a clinical course of life-long pain, disability and early death. Though the childhood death rate of individuals with SCD has drastically fallen due to disease management, transfusion therapy and hydroxyurea, patients with SCD continue to suffer serious morbidity and premature mortality (1). To date, no drugs have been approved that specifically target the underlying mechanism of SCD. A drug that inhibits HbS polymerization in all RBCs has the potential to provide superior efficacy. Because oxyhemoglobin is a potent inhibitor of HbS polymerization, allosteric modification of hemoglobin to increase the proportion of oxyhemoglobin is a promising strategy to achieve inhibition of HbS polymerization in all RBCs (2). By delaying polymerization the irreversible damage done to the RBC membrane can be prevented and thereby the downstream pathophysiology of the disease (3).

In order to fulfill this unmet need, Global Blood Therapeutics (GBT) has developed GBT440, a small molecule allosteric modulator of hemoglobin oxygen affinity for the treatment of SCD. Though GBT440 binds to the N-terminal of the α chain, it allosterically affects the entire RBC as well as the αβ interface. These allosteric effects allow hemoglobin to maintain a population in the deoxyhemoglobin, released state, delay polymerization, reduce cytoplasmic hyperviscosity, improve membrane elasticity and improve deformability.

Materials and Methods

Crystallographic studies

For cryo-electron microscopy and single particle analysis of crystals, extended HbS crystals were prepared and used as described previously (8). Single crystals were grown in 10% buffer in 40% EGT440 using a 21-day soak protocol. X-ray diffraction data were collected at a wavelength of 0.9Å using a Rigaku 4-circle crystallographic diffractometer equipped with a rotating anode (Rigaku, SMART X-3100) using Cu-Kα radiation. Crystals were grown using a 21-day soak protocol. X-ray diffraction data were collected at a wavelength of 0.9Å using a Rigaku 4-circle crystallographic diffractometer equipped with a rotating anode (Rigaku, SMART X-3100) using Cu-Kα radiation.

Structural and Rheological Studies

Structural studies, using crystallography and solution phase NMR, indicate that GBT440 allosterically affects the heme pocket of the β chains

Mechanism of Action

Under physiologic conditions, HbS will form polymers upon deoxygenation. The formation of extensive polymer leads to sickling in the microcirculation. In vitro experiments show that GBT440-modified HbS delays polymerization and thereby delays formation of sickled cells, allowing them to exit the microcirculation and get rehydrated in the large.

GTx011* (GBT440) maintains RBC deformability under hypoxic conditions

GT440 improves SS RBC filterability under hypoxic conditions

GT440 reduces tension required to aspirate SS RBCs under hypoxic conditions

GT440, an anti-sickling compound, has been shown to:
- Allosterically affect the heme pocket of hemoglobin
- Induce a delay in polymerization and reduce cytoplasmic hyperviscosity in a dose dependent manner
- Improve SS RBC deformability under hypoxic conditions

Based on these findings, GBT440 is expected to inhibit the polymerization of deoxygenated HbS, improve RBC deformability and reduce whole blood viscosity in SCD patients.

Conclusions

References

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Global Blood Therapeutics Project Team

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*GTx011 is now being referred to as GBT440

**For more information on the effect of GBT440 on Townes S Mice Model please attend our Oral presentation on Monday (#213)