GTx011, A Novel Agent That Improves Rheological Properties Of Sickle Cell Blood By Increasing Oxygen Affinity For Hemoglobin.

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Introduction
Sickle cell disease (SCD) is an inherited hemoglobinopathy affecting a substantial portion of the African populations and more than 40 million African-Americans (1). It is a genetic disorder characterized by the presence of coiled red blood cells (RBCs) that are the result of a single-point mutation in hemoglobin (Hb) in the β-globin at the 6th position. The hydrophilic glutamic acid is replaced by a hydrophobic valine. This mutation does not alter the oxygen affinity or change the structure of β in the Hb tetramer (2). However, in the T (deoxy) state, Hb has fewer patients (HbB) forms polymers in low oxygen environments. When polymers form, the erythrocytes grow large enough, they can lead to the development of non-deformable RBCs. If the RBCs are further deformed, they can develop into the characteristic sickle shape. The non-deformable RBCs are unable to pass through narrow blood vessels or the capillaries of the lungs. These non-deformable cells lead to the physiologic effects of SCD: anemia, skin rash, and major organ damage, including renal insufficiency and non-functional spleen (3,4).

Though the childhood death rate of individuals with SCD has dramatically fallen due to disease management, transfusion therapy and hydroxyurea, there is no standard treatment for SCD (5). It has been shown that modifying O2 affinities can alter the polymerization dynamics of a manner that may be beneficial in SCD (6,7). However, to date, none of these modifications are approved for use in patients.

In order to fulfill this unmet need, Global Blood Therapeutics has developed a novel series of synthetic compounds that increase the O2 affinity of RBCs, delay polymerization and improve the rheological properties of SSBlood.

Materials and Methods
Determination of oxygen equilibrium curve (EOC) of purified, washed RBCs, and blood
Purified Hb samples (HbA, HbA2, HbF, and HbSS) were isolated using Protosol (GTx) and centrifugation in a 14,000 rpm swing-out rotor and resuspended in 50 mM HEPES buffer pH 7.4 to ensure a red cell concentration of 1.2% by volume (0.7% hematocrit) or presence of compound. 160 μL of RBCs or blood was added to 1 mL of Hb assay buffer at 37°C. Oxygen-equilibrium curves (EOC) were then collected with a Hemo-analyzer (5).

Oxygen Dissociation Assay
Determine the ability of GTx011 to maintain the oxygenated state of hemoglobin (oxyHb) under oxygenated conditions. Purified Hb (0 µM) was incubated for 3 hours at 37°C in the presence or absence of compound in 50 mM phosphate buffer, pH=7.4 in 6-well plate assay. Plates were then placed in a WAVE1600 tissue plate reader (BioTek) and incubation performed at 37°C with 5% CO2 for 2 hours.

Viscosity
The viscosity of treated and control blood was measured using a cone-plate viscometer (RheoStress III). Blood was measured at 37°C, reduced to 30% and then incubated for 30 minutes at room temperature with GTx011 at 100 nM. The reaction mixture was then transferred into a 5 mm gas permeable 24 well plate placed within a humidified chamber (37°C, 4% CO2, 5% O2). Room temperature equilibrated blood samples were run in oxygenated conditions. Data was collected at shear rates ranging from 60 to 475 s⁻¹.

Viscosity

Conclusions
GTx011, an insoluble compound, has been shown to:
- Shift the OEC curve, indicating a higher oxygen affinity than control Hb, RBCs and Blood.
- Maintain the oxyHb state under hypoxic conditions.
- Delay polymerization similar to HbA.
- Reduce the viscosity of deoxygenated SSBlood.
GTx011 is anticipated to improve blood flow by increasing RBC deformability and reducing in vivo sickling events.

References

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For more information on sickling and polymerization results of GTx011 please visit our Oral presentation tomorrow [#316]