GBT021601 Improves the Pathophysiology of Sickle Cell Disease in a Murine Model

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BACKGROUND

Sickle cell disease (SCD) is characterized by hemolytic anemia, vaso-occlusion, and progressive end-organ damage. The underlying mechanism of SCD involves polymerization of tetrameric HbS into higher-order oligomers in the microvasculature, leading to decreased red blood cell (RBC) deformability, morphology, sickling, reduced RBC survival, microvascular obstruction, and clinical complications.1,2

1. Vasoreactivity: A RBC polymerization inhibitor in preclinical models demonstrated increased RBC deformability and decreased sickling in vivo.

GBT021601 is a potent, next-generation HbS polymerization inhibitor with the potential to achieve even higher RBC occupancies in patients with SCD at lower doses and therefore with less adverse burden.

OBJECTIVE

To evaluate the effect of GBT021601, a second-generation HbS polymerization inhibitor, on the pathophysiology of sickle cell disease (SCD) in a murine model.

METHODS

• In vitro: Hb polymerization: GBT021601-modified purified HbS (1.8 µM) was polymerized in a 1:8 HbO2-buffer (pH 7.4) at 37 °C for 24 h as described.1

• In vivo: modification of purified HbS (50 mM HbS, pH 7.4) was incubated with sodium dithionite, followed by a temperature jump to 37 °C to initiate polymerization, and monitored at an optical density of 700 nm.

• Dosing of Transgenic (Tg) mice: mice were dosed with GBT021601 at 20, 40, 75, and 150 mg/kg QD via oral gavage. Blood from individuals with SCD

• Serum erythropoietin (EPO) was measured by enzyme-linked immunosorbent assay using the Hs EPO Quantikine Kit (R&D Systems).

• Complete blood count was determined using a Sysmex XT-2000iV (CDEK Diagnostics). Markers of RBC morphology were analyzed for fluorescence on a BD Accuri C6 cell analyzer.

• HbS sickle hemoglobin, pO2, partial pressure of oxygen; O2eq, calculated Hb saturation; Hb occupancy, with corresponding increases in Hb-O2 affinity.

• Oxygen-scarce cells were analyzed using an Hanks’ buffer containing 95% N2 and 5% CO2 (pH 7.4) immediately fixed in 2% glutaraldehyde/PBS.

RESULTS

• In vitro modification of purified HbS (50 mM HbS, pH 7.4) with GBT021601 followed by deoxygcnation resulted in a concentration-dependent increase in the OEC delay time leading to the onset of sickling (Figure 1A).

• In vitro modification of blood (20% hematocrit) from individuals with SCD with the indicated concentrations of GBT021601 followed by deoxygcnation in a concentration-dependent decrease in the percentage of sickled RBCs (Figure 1B).

• There was a dose-dependent left-shift of the OEC of blood from GBT021601-treated SS mice relative to that of vehicle-treated SS mice (Figure 2A).

• GBT021601-treated SS mice showed a significant reduction in Hb occupancy, with corresponding increases in Hb-O2 affinity in blood of SS mice (Figure 3A).

• Blood from individuals with SCD

• Oxygen-scarce cells were analyzed using an Hanks’ buffer containing 95% N2 and 5% CO2 (pH 7.4) immediately fixed in 2% glutaraldehyde/PBS.

• In vitro modification of purified HbS (50 mM HbS, pH 7.4) with GBT021601 followed by deoxygcnation resulted in a concentration-dependent increase in the OEC delay time leading to the onset of sickling (Figure 1A).

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CONCLUSIONS

GBT021601 was well tolerated, and resulted in reduced Hb in all RBCs. In clinical studies in patients with SCD, voxelotor at 1500 mg daily (QD) dosed over 21 or 42 days to observe changes in the pathophysiology of RBCs, reticulocytes, and Hb over different dosages and dose durations compared to vehicule-washed blood.

GBT021601 improved SCD pathophysiology in a Hb-occur-dose-dependent manner (mean percentage changes are shown, Figure 7).

GBT021601 showed a dose-dependent reduction in the percentage of sickled RBCs (Figure 1B).


Acknowledgments

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Disclosures

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