Pharmacological Increase of Hb-O_2 Affinity With a Voxelotor Analog Does Not Decrease Brain Tissue pO_2 or Limit O_2 Extraction in Brain Tissues of Sickle Cell Mice

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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 is the polymerization of sickle hemoglobin (HbS) that occurs when sickle erythrocytes are deoxygenated in microcirculation, leading to SCD's pathophysiologic features. One of the most devastating complications of SCD occurs in the central nervous system, where overt stroke or repeated silent cerebral infarcts lead to significant physical and neurocognitive consequences.[1-3]
- In SCD, anemia-induced tissue hypoxia is an important factor contributing to SCD morbidity. Low hemoglobin (Hb) levels and increased cerebral blood flow are associated with increased stroke risk, suggesting that anemia-induced tissue hypoxia is an important factor contributing to subsequent morbidity.[4,5]

OBJECTIVES

- To assess the impact of a pharmacologically mediated increase in Hb affinity for oxygen (O_2) on brain tissue oxygenation, blood pressure, heart rate, arterial-venous blood gases, and tolerance to hypoxia using Townes transgenic SCD mice.
- To establish the implication of increased Hb-O_2 affinity in SCD during normoxia and hypoxia.

METHODS

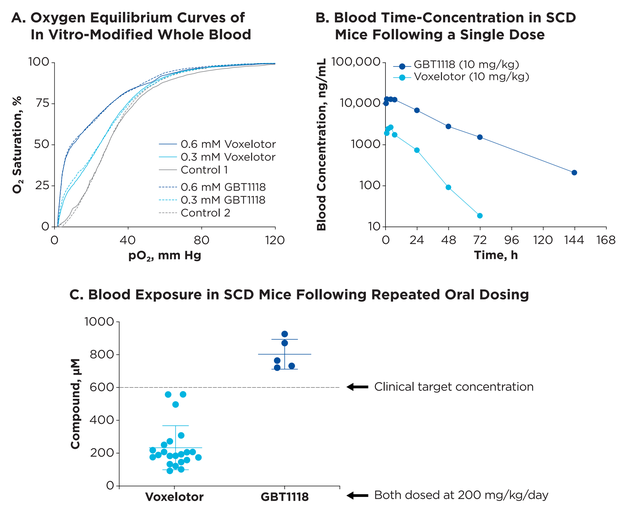
- **Animal Models:** Homozygous Townes (B6; 129-Hba$^{tm1(HBA)Tow}$ Hbb$^{tm2(HBG1,HBB)Tow/Hbb^{tm3(HBG1,HBB)Tow}/J}$) transgenic SCD mice of C57BL/6J background from the Jackson Laboratory (Bar Harbor, ME, USA) were used. Wild-type C57BL/6J mice from the Jackson Laboratory were used as controls in tolerance to hypoxia evaluation.
- **Pharmacological Treatment to Increase Hb-O_2 Affinity:** SCD mice were dosed with 200 mg/kg/day of GBT1118 (a voxelotor analog) or voxelotor, split into 2 doses of 100 mg/kg separated by 7 hours, via oral gavage for 14 days. Vehicle-treated control SCD mice received equal volume of vehicle (0.5% methylcellulose/phosphate buffer pH 7.4/0.01% Tween-80) in an identical dosing schedule. All mice were studied within 1 hour of the last dose.
- **Animal Preparation for Blood Gases:** Mice were anesthetized with ketamine-xylazine. After shaving the neck and cleansing with ethanol 70% and betadine, mice were placed on a warm surgical stage and the carotid artery and jugular vein were catheterized. Arterial and venous catheters were filled with a heparinized saline solution (30 IU/mL) to ensure patency at the time of the experiment. During surgery, animals breathed spontaneously with 40% O_2 to ensure appropriate oxygenation.
- **Systemic Parameters:** Mean arterial pressure (MAP) was measured via a carotid artery catheter, and heart rate (HR) was determined from the pressure (MP 150, Biopac Systems, Santa Barbara, CA, USA). MAP and HR were continuously monitored for all animals.
- **Hematological Measurements:** Two microhematocrit tubes collected from arterial and venular catheters were used to determine: (1) arteriolar and venular blood gases (ABL-90 FLEX, Radiometer, Denmark); (2) hematocrit (centrifugation); and (3) total Hb content, determined spectrophotometrically from a single drop of blood (B-Hemoglobin, Hemocue, Stockholm, Sweden). Arterial and venous blood gas samples were taken at normoxia and after 15 minutes of exposure to 10% O_2 in one set of experiments.
- **Blood O_2 Equilibrium Curve:** Hb-O_2 equilibrium curves were obtained by deoxygenation of O_2-equilibrated samples in Hemox buffer at 37°C, using a Hemox Analyzer (TCS Scientific, New Hope, PA, USA).
- **Brain Measurements:** Brain cortical tissue O_2 tensions in a cranial window were assessed directly with O_2-specific microelectrodes and indirectly with hypoxyprobe staining (pimonidazole) during normoxia and after 15 minutes of exposure to 10% O_2 in another set of experiments.
- **Tolerance to Hypoxia:** Mice were exposed to decreasing fractions of inspired O_2 (FiO_2) in steps (21% O_2 [normoxia], 15% O_2, 10% O_2, and 5% O_2) and maintained for 15 minutes at each FiO_2. However, mice that were exposed to 5% O_2 were evaluated for 30 minutes in a different set of experiments. Mice were considered non-survivors if MAP fell below 35 mm Hg for more than 60 seconds, after which the animals were sacrificed.

Abbreviations: FiO_2, fractions of inspired O_2; Hb, hemoglobin; HbS, sickle hemoglobin; HR, heart rate; MAP, mean arterial pressure; O_2, oxygen; PK, pharmacokinetics; pO_2, partial pressure of oxygen; SCD, sickle cell disease.

RESULTS

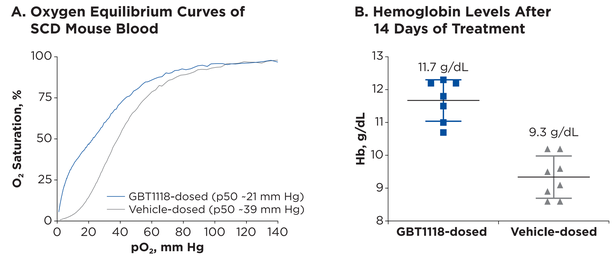
- In vitro modification of blood samples (20% hematocrit) with voxelotor or GBT1118 resulted in a dose-dependent left-shift of the oxygen equilibrium curves to the same degree by the 2 compounds. Results indicate that GBT1118 increases Hb-O_2 affinity with similar potency to voxelotor (**Figure 1A**).
- Time-concentration profiles following single oral doses (10 mg/kg) in SCD mice indicate that GBT1118 has higher exposure and longer half-life compared with voxelotor in SCD mice (**Figure 1B**).
- Blood exposures following repeated oral dosing (200 mg/kg/day) of GBT1118 or voxelotor for 14 days in SCD mice showed that voxelotor did not consistently achieve the clinical target concentration of 600 µM in SCD mice. In contrast, GBT1118 consistently achieved the clinical target blood exposure and was subsequently used to assess the impact of increased Hb-O_2 affinity on blood gases, oxygen delivery, brain oxygen tension, and tolerance to hypoxia in SCD mice (**Figure 1C**).

Figure 1. GBT1118 Increases Hb-O_2 Affinity With a Potency Similar to Voxelotor but Showed Greater Improvements in PK Properties Compared With Voxelotor



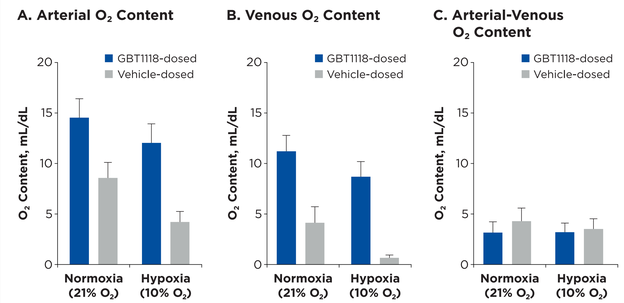
- Oxygen equilibrium curves of blood from SCD mice repeatedly dosed with GBT1118 (200 mg/kg/day for 14 days) indicate an increase in Hb-O_2 affinity as evidenced by the left-shift of the curves (**Figure 2A**).
- Repeated oral dosing of GBT1118 (200 mg/kg/day for 14 days) also resulted in a 2.5 g/dL increase in total Hb compared with the vehicle-treated mice (**Figure 2B**).

Figure 2. Repeated Oral Dosing of GBT1118 in SCD Mice Increased Hb-O_2 Affinity and Hb Levels



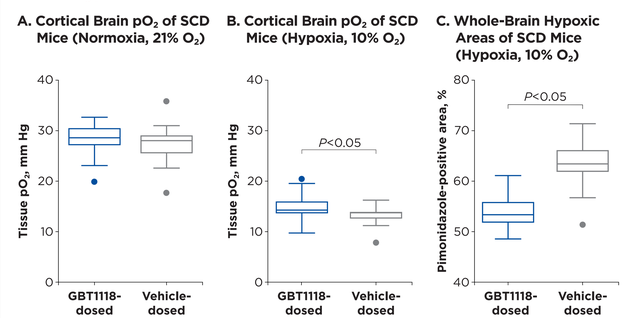
- Blood gas analysis shows that the pharmacological increase of Hb-O_2 affinity:
  - Resulted in increased arterial O_2 content under normoxic (21% O_2) and hypoxic (10% O_2) conditions (**Figure 3A**).
  - Resulted in increased venous O_2 content under normoxic (21% O_2) and hypoxic (10% O_2) conditions (**Figure 3B**).
  - Did not limit systemic O_2 extraction in SCD mice (**Figure 3C**).

Figure 3. The Pharmacological Increase of Hb-O_2 Affinity Resulted in Increased Oxygen Delivery and Did Not Limit Oxygen Extraction in SCD Mice



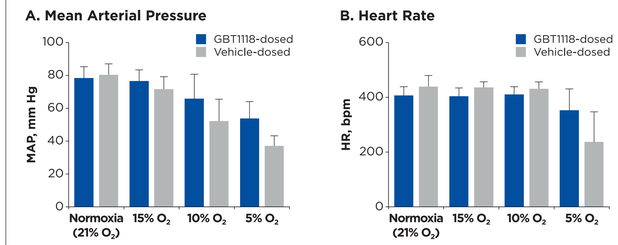
- Brain oxygenation measurements indicate that the pharmacological increase of Hb-O_2 affinity:
  - Did not decrease brain tissue pO_2 under normoxia (21% O_2; **Figure 4A**).
  - Significantly increased brain tissue O_2 tension under hypoxic conditions (10% O_2; **Figure 4B**).
  - Significantly reduced brain tissue hypoxia during exposure of SCD mice to 10% O_2 hypoxia (**Figure 4C**).

Figure 4. The Pharmacological Increase of Hb-O_2 Affinity Reduced Brain Tissue Hypoxia in SCD Mice



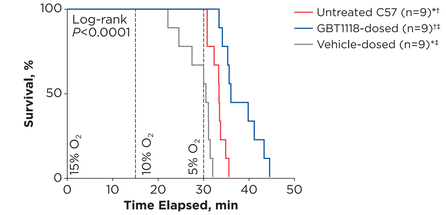
- The pharmacological increase of Hb-O_2 affinity in SCD mice maintains MAP and HR during exposure to progressive hypoxia.
  - During exposure to severe hypoxia (5% O_2), pharmacological increase in Hb-O_2 affinity sustained MAP in GBT1118-dosed SCD mice relative to vehicle-dosed SCD mice (**Figure 5A**).
  - During exposure to 5% O_2, pharmacological increase in Hb-O_2 affinity preserved HR in GBT1118-dosed SCD mice relative to vehicle-dosed SCD mice (**Figure 5B**).

Figure 5. The Pharmacological Increase of Hb-O_2 Affinity Is Cardio-Protective During Exposure to Hypoxia



- GBT1118-dosed mice exposed to decreasing FiO_2 at 15-minute intervals demonstrated improved tolerance to hypoxia compared with untreated C57 and vehicle-dosed mice (**Figure 6**).

Figure 6. The Pharmacological Increase of Hb-O_2 Affinity Improved Tolerance of SCD Mice During Exposure to Severe Hypoxia



Note: Mice were considered non-survivors if MAP fell below 35 mm Hg for more than 60 seconds. *$P<0.01$ vs GBT1118-dosed. †$P<0.01$ vs vehicle-dosed. ‡$P<0.01$ vs untreated C57.

CONCLUSIONS

- Under hypoxic conditions, the pharmacological increase of Hb-O_2 affinity improves survival, cardiovascular function, and brain oxygenation in SCD mice.
- A pharmacological increase of Hb-O_2 affinity does not impair oxygen extraction by tissues including the brain under either normoxia or hypoxia in SCD mice.
- A pharmacological increase of Hb-O_2 affinity led to increased Hb content, O_2 carrying capacity, and O_2 delivery in SCD mice.

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Disclosures

- **Kobina Dufu, Xinchun Zhang, Peter Rademacher, Carsten Alt, Donna Oksenberg:** Global Blood Therapeutics, employees and equity ownership. **Alfredo Lucas, Cynthia Rodrigues Muller, Alexander T. Williams, Pedro Cabrales:** Global Blood Therapeutics, research funding.

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Presented at: 61st ASH Annual Meeting and Exposition; December 7-10, 2019; Orlando, FL, USA

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