GBT440 Demonstrates High Specificity for Red Blood Cells in Nonclinical Species

Athiwat Hutchaleelaha, Mira Patel, Abel Silva, Donna Oksenberg, Brian Metcalf

Global Blood Therapeutics, Inc., South San Francisco, CA 94080

Introduction

Sickle cell disease (SCD) is caused by a point mutation in the β-globin gene leading to production of hemoglobin S (HbS), which polymerizes upon deoxygenation with subsequent formation of sickled red blood cells (RBCs). GBT440 modulates O_2 affinity of hemoglobin (Hb) by binding to the N-terminal α chain of Hb via a reversible Schiff base. We previously demonstrated that GBT440 prevented sickling of RBCs from sickle cell patients *in vitro*. Also, in a murine model of sickle cell disease (Townes SS mice), GBT440 prevented *ex vivo* sickling of RBCs and prolonged RBC half-life. In this presentation, ADME profiles and pharmacokinetic/pharmacodynamic (PK/PD) relationship in nonclinical species are described.

Methods

Pharmacokinetic study

Species	N IV/PO	IV Dose (mg/kg)	PO Dose (mg/kg)	Blood and Plasma Sample Collection Period
Sprague-Dawley Rat	4/4	1.6	7.2	0-96 hr
Beagle Dog	6 (IV crossover to PO)	1	2.5	0-96 hr
Cynomolgus Monkey	3 (IV crossover to PO)	1	4.25	0-96 hr

PK/PD Correlation in Mice

Mice were given an oral (PO) dose of 30, 50 and 500 mg/kg GBT440 (n = 3/dose). In addition to GBT440 blood concentration analysis, blood was also collected for hemoximetry analysis at 4 hr postdose to determine the PD effect (change in hemoglobin oxygen affinity).

Analysis of Blood and Plasma GBT440 Concentrations

Blood and plasma samples were analyzed for GBT440 concentrations using LCMS. The analytical range was 50 to 100,000 ng/mL for blood and 10 to 20,000 ng/mL for plasma samples.

Hemoximetry

Blood samples were kept at 4°C until analysis. The hematocrit (Hct) for each individual sample was determined prior to analysis with a Hemox Analyzer (TCS Scientific Corporation, New Hope, PA). After appropriate dilution in buffer, the samples were transferred to the hemoximeter sample chamber where they were first saturated with compressed air for 20 minutes and then deoxygenated with pure nitrogen. The absorbance at wavelengths that correspond to the isosbestic point (570 nm) and deoxy Hb (560 nm) was recorded as a function of the sample O2 tension (pO2). During deoxygenation the pO2 and %O2 saturation are recorded to obtain an OEC as well as a p50 value.

Rat Mass Balance Study

A mass balance study of ^{14}C-GBT440 (10 mg/kg; 150 µCi/kg PO) was conducted in 3 male Sprague-Dawley rats to determine route of elimination of GBT440. Urine, feces and expired air samples were collected serially up to 240 hours post-dose. Animal carcasses was also collected at the end of the study. After appropriate processing, all samples were analyzed for radioactivity with a liquid scintillation counter.

Rat QWBA

A quantitative whole body autoradiography study (QWBA) to determine tissue distribution of GBT440 in pigmented and non-pigmented rats was conducted following an oral dose of ^{14}C-GBT440 (10 mg/kg; 150 µCi/kg). One non-pigmented rat (Sprague-Dawley)/time point was prepared for QWBA at 0.5, 4, 8, 24, and 72 hr. One pigmented rat (Long Evans)/time point was prepared for QWBA at 0.5, 4, 8, 24, 48, 72, 168, 336 and 672 hr.

Predicted vs. Actual Human PK Profiles

PK parameters in rat, dog, and monkey were used to predict PK parameters in human using simple allometric scaling method, assuming a one compartment model. Blood concentration-time profile was simulated assuming an absorption rate constant (K01) of 1.15 1/hr and oral bioavailability of 37% (similar to dog). Actual human PK data were obtained from the ongoing human clinical trial, GBT440-001 (ClinicalTrials.gov NCT02285088).

Results and Discussion

Figure 1. Blood and Plasma Concentration-time Profiles of GBT440 in Rat, Dog, and Monkey Following IV/PO Administration

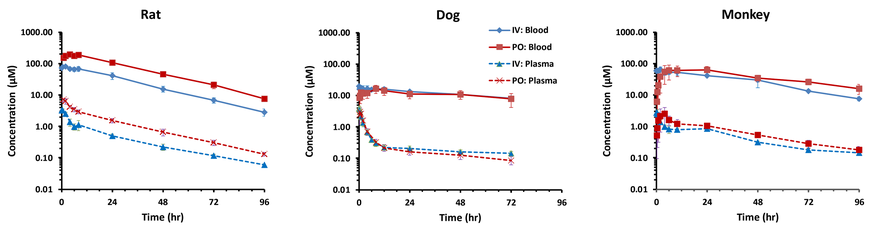


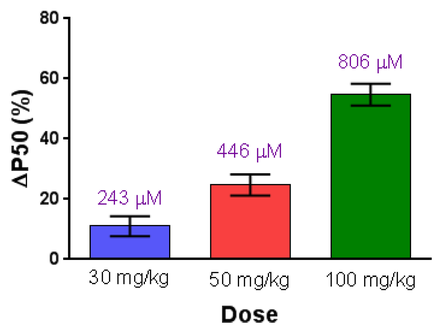
Table 1 Pharmacokinetic Parameters of GBT440 in Rat, Dog, and Monkey Following IV/PO Administration

Species	Matrix	IV Dose (mg/kg)	CLs (mL/min/kg)	Vss (L/kg)	T½ (hr)	PO Dose (mg/kg)	Cmax (µg/mL)	Oral Bioavailability (%F)	Blood/Plasma Ratio	RBC/Plasma Ratio
Rat	Blood	1.6	0.031	0.049	19.1	7.2	71.2	59.8	69.0	152
	Plasma		1.80	2.78	21.8		2.41			
Dog	Blood	1	0.031	0.171	66.0	2.5	5.56	36.6	74.4	164
	Plasma		1.29	8.45	93.5		1.04			
Cyno	Blood	1	0.016	0.041	28.8	4.25	25.2	36.1	70.9	156
	Plasma		0.943	2.34	28.8		0.871			

Pharmacokinetics (Figure 1; Table 1)

Following intravenous (IV) and oral (PO) administrations, GBT440 quickly partitions into the RBC with a high specificity with blood/plasma ratio of ~70:1 which corresponded to a RBC/plasma ratio of ~150:1. Volume of distribution (Vss) was small in whole blood (0.041–0.171 L/kg) but much larger in plasma (1.44–8.45 L/kg) indicating that RBCs are a reservoir of GBT440. Systemic clearance (CLs) was low in both blood (0.016–0.113 mL/min/kg) and plasma (0.943–3.16 mL/min/kg) indicating that GBT440 was mostly bound to hemoglobin and only a small fraction of unbound GBT440 re-distributed into the plasma and was available for clearance. Terminal elimination half-life (t1/2) was similar between whole blood and plasma for each species which suggests that GBT440 did not bind to other component(s) in the plasma that would prolong its elimination. GBT440 was well absorbed and absolute oral bioavailability (%F) ranged from 33% to 70% in the three species.

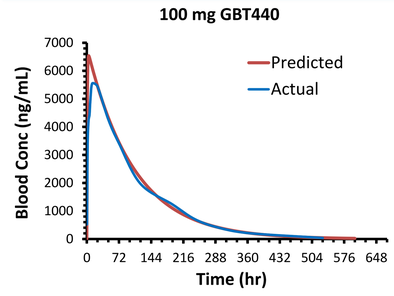
Figure 2. PK/PD Correlation in Mice



PK/PD Correlation (Figure 2)

To further understand the relationship of pharmacokinetic to the pharmacodynamic effect (change in Hb-O_2 affinity, based on changes in the p50 determined from the oxygen equilibrium curve), mice were given an oral dose of GBT440 at 30, 50 and 500 mg/kg. Blood concentrations following 30, 50 and 100 mg/kg at 4 hr were 243, 446, and 806 µM, which resulted in changes in p50 of 11%, 25% and 55%, respectively. These data indicate that GBT440 demonstrates dose-dependent and linear pharmacokinetics and elicits an ex vivo dose dependent increase in Hb-O_2 affinity following increasing dosage to mice.

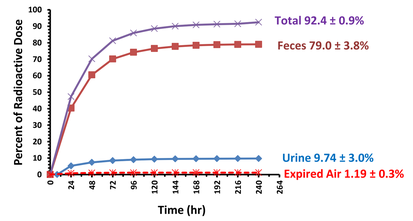
Figure 3. Actual vs. Predicted PK of GBT440 in Healthy Subjects



Actual vs. Predicted PK in Healthy Subject (Figure 3)

Based on PK data from animal species, the PK profile of GBT440 in humans following 100 mg PO was predicted using a simple allometric scaling technique. The predicted PK profile was highly concordant with actual data from healthy subjects (study GBT440-001) for Cmax (5.73 µg/mL), AUC(0-∞) (641 µg*hr/mL) and T½ (72 hr), which suggested that the disposition kinetics of GBT440 in humans were consistent to that in animals.

Figure 4. %Dose Recovered Following a Single PO Dose of 10 mg/kg (150 µCi/kg) ^{14}C-GBT440

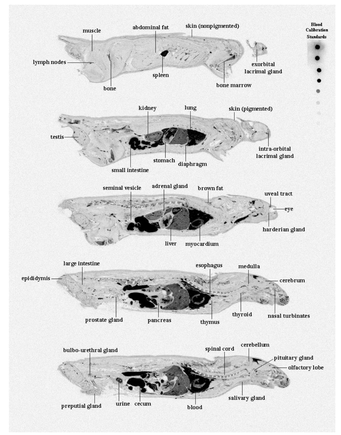


Rat Mass Balance Study (Figure 4)

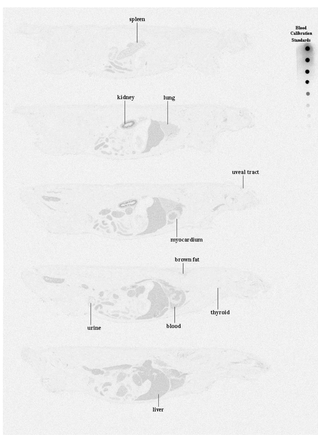
^{14}C-GBT440-derived radioactivity demonstrated that GBT440 was well absorbed and rapidly excreted after oral administration. By 240 hours postdose, mean values of 79.0 ± 3.86 and 9.74 ± 3.02% of the administered radioactivity were excreted in feces and urine, respectively. The mean overall recovery of radioactivity was 92.4 ± 0.875%. Metabolism via both Phase I and Phase II pathways was the major route of elimination of GBT440. These data indicate that despite its high affinity binding with Hb, GBT440 could be released from the hemoglobin complex and completely eliminated from the body.

Figure 5. QWBA in Rat at 4 hr (Panel A) and 168 hr (Panel B) Following a Single PO Dose of 10 mg/kg (150 µCi/kg) ^{14}C-GBT440

Panel A: 4 hr



Panel B: 168 hr



Quantitative Whole Body Autoradiography (representative data, Figure 5)

Following a single oral dose at 10 mg/kg of ^{14}C-GBT440, distribution trends of drug-derived radioactivity in pigmented rats were generally similar to those seen in the nonpigmented rats. Blood radioactivity was highest at 8 hr (56300 ng equivalent/g), and then declined to 42.7 ng equivalent/g by 672 hr postdose. In plasma, the concentration of radioactivity was highest at 4 hr (4900 ng equivalent/g), and then declined to BLQ by 336 hr postdose. Blood:plasma ratio was high which suggest preferential association of drug-related radioactivity with the cellular components of blood. Most tissues had radioactivity concentration at 8 hr postdose. The matrices with the highest concentration of drug-related radioactivity corresponded to contents in the gastrointestinal tract. ^{14}C-GBT440-derived radioactivity was not selectively associated with melanin-containing tissues.

Conclusions

- GBT440 showed high specificity for red blood cells with RBC/plasma ratio of ~150:1.
- Pharmacokinetic data showed
  - GBT440 was well absorbed in all animal species tested
  - Dose-dependent and linear pharmacokinetics
  - Half-life which is suitable for once daily dosing (human data, ~72 hr).
- Pharmacodynamic data showed good correlation between GBT440 blood concentration and an increase in hemoglobin O_2 affinity, as measured via hemoximetry
- Metabolism is the major route of elimination involving both Phase I and Phase II metabolism pathways, with complete elimination from the body.
- GBT440 distributes to hematopoietic tissues as expected for a molecule whose target is hemoglobin, including blood, spleen, liver and bone marrow.

These data support that GBT440 preferentially partitions to RBCs, binds specifically to Hb, modulates Hb-O_2 affinity and has a half-life suitable for once daily dosing. GBT440 is in clinical trials for the treatment of sickle cell disease.