GBT440 Increases Hematocrit and Improves Biventricular Function in Berkeley Sickle Cell Disease Mice

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Sickle cell disease (SCD) is a hereditary blood disorder affecting millions of people in which red blood cells (RBCs) become sickled and lyse easily driven by polymerization of hemoglobin. Chronically, SCD causes anemia and biventricular dysfunction. GBT440 is an experimental treatment for SCD that prevents hemoglobin polymerization. We hypothesized that 17-month-old Berkeley SCD mice treated with GBT440 would have increased hematocrit (Hct) and better biventricular function compared to vehicle treated SCD mice. Our results demonstrate that 3 weeks of GBT440 treatment eliminated chronic anemia, increased left ventricular ejection fraction (LVEF) and stroke volume index, and improved right ventricular function. Overall, our findings support a therapeutic effect of GBT440 in vivo in a small animal model of SCD. Next steps in investigating mechanisms of improved cardiac function are warranted. [DOI: 10.1115/1.4049079]

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Manuscript received April 27, 2020; final manuscript received August 5, 2020; published online December 10, 2020. Assoc. Editor: Katherine Yanhang Zhang.

Introduction

Sickle cell disease (SCD) is a hereditary disorder that affects millions of people worldwide and is estimated to affect 300,000 newborn children per year [1,2]. In the U.S. in 2013, there were 90,140 primary hospital admissions for SCD at a total direct cost of an estimated $762 million [3]. SCD is characterized by a point mutation in the β-globin gene resulting in the incorporation of the amino acid valine instead of glutamic acid [4]. This results in the primary consequence of SCD, which is the polymerization of hemoglobin in low oxygen conditions, giving red blood cells (RBCs) a sickled morphology, lower oxygen carrying capacity, and susceptibility to lysis, all of which result in anemia [4,5]. There is demand for new treatments for SCD that directly treat the polymerization of hemoglobin and improve quality of life for SCD patients, as well as decrease hospitalization costs.

Sickled RBCs with a tenfold shorter lifespan frequently lyse, resulting in the release of cell-free hemoglobin [6]. Cell free hemoglobin readily binds nitric oxide (NO), which is a potent vasodilator and inhibitor of mast cell release of pro-inflammatory cytokines [7]. Thus, increased hemolysis lowers Hct, increases vasoconstriction, and increases inflammation. These mechanisms increase systemic and pulmonary vascular resistance and, in combination with decreased oxygen delivery to the myocardium, cause biventricular dysfunction [5–7].

Preventing the polymerization of hemoglobin may reduce all-cause morbidity and mortality in SCD. GBT440 inhibits hemoglobin polymerization by binding covalently and reversibly via a Schiff-base to the N-terminal valine of one of the α-chains, increasing the affinity of hemoglobin for oxygen [2]. In vitro, 2 mM of GBT440 caused a 45% reduction in the percentage of sickled RBCs in blood from patients with SCD [8]. Patients with SCD treated with GBT440 experienced a 0.8 g/dL increase in hemoglobin concentration whereas SCD patients treated with placebo decreased hemoglobin concentration by 0.5 g/dL after 28 days [9]. However, the ability of GBT440 to restore biventricular function in SCD remains unknown.

To address this knowledge gap, we tested the ability of GBT440 to reverse anemia and improve biventricular function in a widely used mouse model for SCD, the Berkeley mouse, which expresses exclusively human sickle hemoglobin [10]. We hypothesized that 17-month-old Berkeley SCD mice with moderate pulmonary hypertension treated with GBT440 would have increased Hct (due to decreased hemolysis) and better left and right ventricular function compared to vehicle treated SCD mice.

Methods

Animals. Ten 17-month-old male Berkeley SCD mice were used (The Jackson Laboratory); five were treated with GBT440 (120 mg/kg dissolved in DMSO) via once daily intraperitoneal injections for 3 weeks and the remaining five were treated with DMSO. Previous studies in mice have shown a positive effect of GBT440 on hematological variables in as little as 10 days [2], which was extended here to assess cardiac effects. All mice were allowed to eat and drink *ad lib* and experienced a 12-h light-dark cycle. All procedures were approved by the UW-Madison Institutional Care and Use Committee.

Echocardiographic Measurements. Prior to GBT440 treatment, all mice underwent noninvasive hemodynamic measurements performed via transthoracic echocardiography using a dedicated animal Visual Sonics Vevo 770 ultrasonograph (VisualSonics, Toronto, ON, Canada) [11]. Mice were ventilated with 1% isoflurane and 99% O_2 on a heated platform to preserve normal body temperature. Echocardiography was performed using established LV M-Mode, mitral valve, aortic valve, tricuspid valve, and pulmonary valve (PV) protocols. Parameters were averaged over three consecutive cardiac cycles. These measurements were

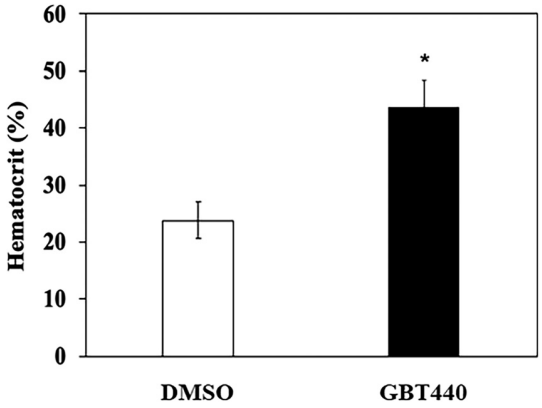

Fig. 1 Average hematocrit of DMSO- and GBT440-treated mice ($p = 0.009$). Data are displayed as mean ± SE. Asterisk denotes $p < 0.05$. $N = 5$ per group.

repeated on the GBT440 treated mice at the end of the 3-week treatment.

Ex Vivo Measurements. After euthanasia, heparinized blood was centrifuged at 500 g for 5 min, and Hct was measured manually via a card reader. Right ventricle (RV) free wall and left ventricle (LV) free wall plus septum were weighed to calculate the Fulton index, a measure of RV hypertrophy.

Statistical Analysis. All data are presented as mean ± standard error (SE). To analyze statistical significance, 2-tailed independent or paired Student's *t*-tests were used. $P < 0.05$ was considered a statistically significant difference.

Results

Compared to DMSO treated mice, three weeks of GBT440 significantly increased Hct (Fig. 1) such that the chronic anemia was effectively eliminated (Hct in age-matched C57BL/6 male mice, one of the 5 background strains of Berkeley mice, is ∼37% [12]; Hct in 1 to 6 month old male and female littermate controls of the Berkeley mice is ∼42% [10]). Echocardiography demonstrated that three weeks of treatment increased left ventricular ejection fraction (LVEF) and stroke volume index (stroke volume divided by body weight), as well as the pulmonary valve velocity time integral (PV VTI) (Fig. 2). No difference was found in RV mass comparing GBT440 treatment (mean = 40 mg, SE = 2) to DMSO treatment (mean = 40 mg, SE = 2.7) ($p = 0.77$). Additionally, no difference was found in the mass of the LV free wall plus septum comparing GBT440 treatment (mean = 150 mg, SE = 7.8) to DMSO treatment (mean = 160 mg, SE = 1.4) ($p = 0.24$). The Fulton Index was also not significantly different comparing GBT440 treatment (mean = 0.28, SE = 0.02) to DMSO treatment (mean = 0.26, SE = 0.02) ($p = 0.63$). There was no difference in body mass before treatment nor at euthanasia between treatment groups. Previous phenotyping of the Berekeley strain (males and females, 1 to 6 months of age) have demonstrated cardiac hypertrophy in the SCD mice compared to wildtype littermate controls (heart weight: 0.18 g versus 0.14 g; $p = 0.02$) with no difference in body weight [10].

Discussion

The improvement in biventricular function after 3 weeks of GBT440 treatment in an established animal model of SCD is a significant and novel finding. We observed increases in both LV ejection fraction and stroke volume index after GBT440 treatment, indicating improved LV contractile efficiency. PV VTI was also increased by GBT440 treatment, which correlates with increased RV function [13,14]. The improvement in both LV and RV function is likely due to the nearly doubled Hct in the GBT440 treated group, which complements previous findings of increased RBC count in human SCD patients with GBT440 [8].

In addition to the improved delivery of oxygen to the myocardium enabled by the elimination of chronic anemia, biventricular function may have been improved via reduced inflammation and vasoconstriction. Frequent hemolysis of SCD RBCs results in the release of hemoglobin into the vasculature, which reduces the bioavailability of NO, a potent vasodilator and inhibitor of inflammatory cytokine release [5–7]. The ability of GBT440 to increase Hct suggests that GBT440 reduced hemolysis and thus reduced NO scavenging. Reduced NO scavenging should promote vasodilation and downregulate the release of pro-inflammatory factors [7].

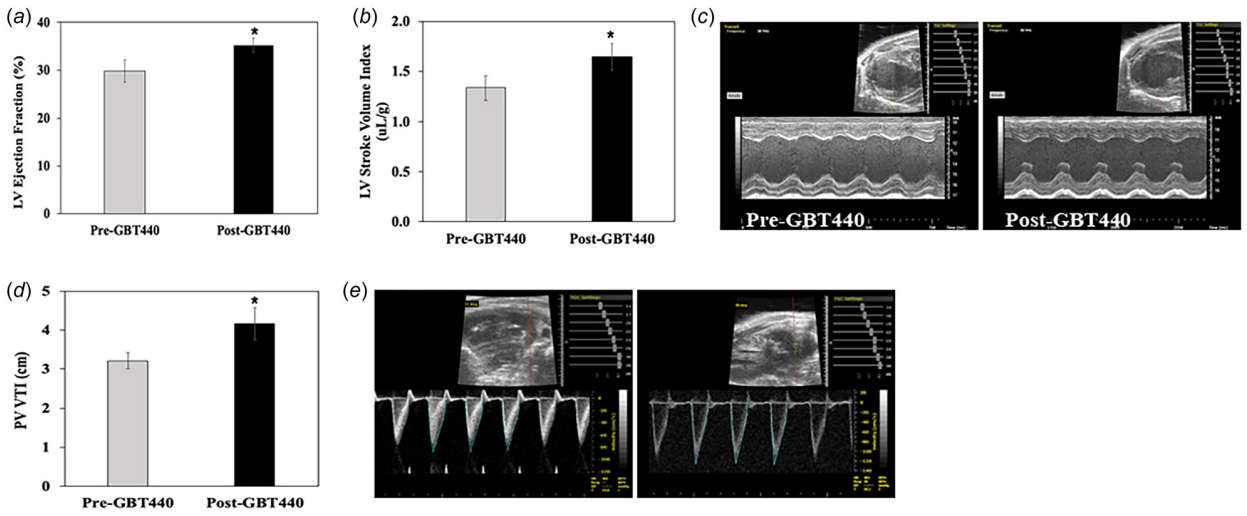

Fig. 2 Effects of GBT440 treatment on biventricular function. (*a*) Average LV ejection fraction pre- and post-GBT440 treatment ($p = 0.02$). (*b*) Average LV stroke volume indexed to body weight pre- and post-GBT440 treatment ($p = 0.014$). (*c*) Raw LV echo trace for one GBT440 treated mouse pre- and post-GBT440 treatment with time (ms) on the *x*-axis and distance (mm) on the *y*-axis. (*d*) Average PV VTI pre- and post-GBT440 treatment ($p = 0.019$). (*e*) Raw RV echo trace for one GBT440 treated mouse pre- and post-GBT440 treatment with time (ms) on the *x*-axis and velocity (cm/s) on the *y*-axis. For (*a*), (*b*), and (*d*), data are displayed as mean ± SE. Asterisk denotes $p < 0.05$. $N = 5$ per group.

Another potential mechanism for improved biventricular function is reduced accumulation of thromboemboli in the heart with GBT440 treatment. Histological evidence of cardiac "microinfarcts" has been found in Berekeley SCD mice but not wildtype littermate controls [10]. If GBT440 prevention of hemoglobin polymerization reduces thromboemboli, the reduced rate of damage accumulation may permit partial recovery. Pre-existing damage likely contributes to the low LVEF found here (35%) even after 3 weeks of treatment.

To further explore the impact of GBT440 treatment on the systemic and pulmonary circulations, which represent the afterload to the LV and RV, respectively, invasive measurements of systemic and pulmonary vascular pressures were obtained in two mice per group (DMSO-treated and GBT440-treated). In brief, after anesthetization with urethane (2 mg/g body weight), mice were intubated and ventilated with a tidal volume of 225 μL and respiratory rate of 200 breaths/min of room air while a 1.2 F pressure transducer (Scisense, Inc., London, ON, Canada) was inserted into the right carotid artery and advanced to the aorta to monitor systemic arterial pressures and a 1.2 F pressure–volume catheter (Scisense, Inc.) was inserted into the superior vena cava and advanced into the RV, as described previously [15]. With the caveat that statistical analysis was not possible, we observed decreased mean aortic pressure in the GBT440 treated group (mean = 69 mmHg, SE = 3) compared to the DMSO treated group (mean = 85 mmHg, SE = 5) and decreased RV systolic pressure in the GBT440 treated group (mean = 24 mmHg, SE = 3.9) compared to the DMSO treated group (mean = 32 mmHg, SE = 2.9).

Despite the improvement in biventricular function found here with treatment, and the suggestion of decreased biventricular afterload with treatment, there were no differences in RV or LV plus septum weights. That is, the cardiac hypertrophy previously reported in these SCD mice [10] persisted with 3 weeks of treatment. Together with the low LVEF noted above, this finding suggests that improved function is not occurring via structural myocardial remodeling and repair.

There are three main limitations to this work. First, due to the use of older (17-month-old) and somewhat fragile mice in this study, invasive catheterization measurements could not be performed on all animals. Second, we did not collect data in littermate wildtype controls for the SCD mice, which limits our ability to quantify the degree of recovery in treated SCD mice. Third, we only used male mice. However, we do not expect sex differences in the effect of GBT440 treatment since RBC sickling in SCD is not sex dependent.

In conclusion, we found 3 weeks of GBT440 treatment eliminated chronic anemia and improved biventricular function in an established mouse model of SCD. The distinction between cardiac and vascular effects of this treatment, as well as the mechanisms and degree of recovery, require further investigation.

Acknowledgment

We would like to thank Allison Rodgers for her echocardiographic expertise. Funding from NIH R21HL129047 (NC) and F31HL128088 (DS), as well as the UW-Madison Hilldale Fellowship (RG) is gratefully acknowledged.

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