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Transgenic sickle cell trait mice do not exhibit abnormal thermoregulatory and stress responses to heat shock exposure

Yifan Chen *, Aminul Islam

Department of Military and Emergency Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, USA

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ABSTRACT

There remains controversy over whether individuals with sickle cell trait (SCT) are vulnerable to health risks during physical activity in high temperatures. We examined thermoregulatory and stress-related responses to heat exposure in SCT and wild-type (WT) mice. No significant differences in core temperature (Tc) were observed between SCT and WT mice during heat exposure. There was no correlation between peak Tc during heat exposure and levels of hemoglobin S in SCT mice. Basal levels of circulating inflammatory and stress-related markers were not significantly different between SCT and WT mice. Although heat exposure caused significant increases in plasma interleukins 1β and 6, and 8-isoprostane in SCT and WT mice, no differences were found between SCT and WT mice with similar thermal response profiles during heat exposure. SCT mice had significantly higher expression of heat shock protein 72 in heart, liver and gastrocnemius muscle than WT mice under control and post-heat conditions. In conclusion, there is neither thermoregulatory dysfunction nor abnormal stress-related response in SCT mice exposed to moderate heat. The hemoglobin variant in mice is associated with altered tissue stress protein homeostasis.

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1. Introduction

Sickle cell trait (SCT), which affects about 1 in 12 African Americans, is often viewed as a benign condition. There is long-standing controversy over whether individuals with SCT are more vulnerable to injury during physical challenges than non-SCT persons [1–4]. Screening for SCT has long been used in professional sports and military recruits, and more recently in college sports as a precautionary measure, but its legitimacy remains contested among both clinicians and scientists [5–7]. Concerns have been raised over potentially unfair precautions or limitations on individuals with SCT that may stigmatize large numbers of people, both socially and economically. Research on SCT has been conducted based primarily on clinical case studies. To date, few studies have examined physiological and biochemical effects of exposure to high temperatures on SCT carriers.

Over the last two decades, several transgenic mouse models that express human hemoglobin S (HbS) have become available [8,9]. These mice that heterozygously express human βS globin gene provide a unique opportunity to experimentally investigate the physiological characteristics associated with SCT. The objective of this study was to determine whether physiological homeostasis is altered locally and systemically in SCT mice under basal and acute heat stress conditions, compared to wild-type (WT) mice. We examined thermoregulatory activities and stress-related markers in tissues and plasma in SCT and WT mice in response to acute heat exposure.

2. Materials and methods

2.1. Animals

All procedures performed on animals were approved by the Uniformed Services University Institutional Animal Care and Use Committee. Mice (male, 8–10 weeks of age when experiments began) were purchased from The Jackson Laboratory (Bar Harbor, ME). The SCT mice were obtained by breeding males exclusively expressing human α- and β^S- transgene globins with hemizygous females expressing mouse β globin gene. They were backcrossed to C57BL/6J one generation at The Jackson Laboratories and had a background of >75% C57BL/6 genomes. The most widely used inbred strain C57BL/6J, which is listed as the "approximate" control for the SCT strain by the supplier, was used as WT controls in this study. No abnormalities or organ injuries were detected in the SCT mice used in this study by the current supplier. This study included four groups of mice: control SCT ($n = 9$), heat-exposed SCT ($n = 16$), control WT ($n = 15$) and heat-exposed WT ($n = 22$). The mice of the control groups were not exposed to heat. All mice were maintained in conventional animal facilities (~22 °C) with ad libitum food and water at the Uniformed Services University Laboratory Animal Medicine facility.

We measured body core temperature (Tc) and locomotor activity (La) using a telemetry system (Mini Mitter Corp, Bend, OR). All experimental

* Corresponding author at: Department of Military and Emergency Medicine, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814, USA.
E-mail address: yifan.chen@usuhs.edu (Y. Chen).

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mice were surgically implanted with a transponder, Model G2 E-Mitter, as described previously [10]. At least 2 weeks were allowed for recovery. At the time of the experimental protocols, all mice were healthy as evidenced by body weight gains (≥pre-surgical levels), normal activity and no sign of infection.

2.2. Experimental procedure

Heat tests were conducted in an environmental chamber (Model 3950, Thermo Forma, Marietta, OH) in the mornings (~9:00 AM–12:00 PM). Mice were placed in the chamber (~22 °C, relative humidity ~22–34%) for at least 4 h on one occasion ~5 days before experimentation. One day before heat tests, mice were housed individually with fresh bedding in the chamber. Heat exposures began the following morning after stable baseline data were obtained. Food and water were removed from cages before heat exposure. The heat protocol used in this study was described previously [10]. Briefly, heat exposure was terminated at core temperature (Tc) of ~42.4 °C or 3 h into heat exposure, whichever happened first. It took ~1 h of heating to reach a predetermined chamber temperature of 39.5 °C. Mice were housed individually and real-time Tc and La were recorded continuously during experiments. Each animal underwent two heat tests separated by an interval of at least 7 days [10] to allow sufficient time for heat stress to recover [11]. We used this design to ensure the appropriate health status of each mouse and minimize experimental variations. The first heat test was used to screen for mice with severe hyperthermia (Tc > 42.4 °C) and extreme physical activity to be excluded from further experiments. Our inclusion criteria also required each mouse to have a normal weight gain between the two heat tests.

2.3. Collection and processing of blood and tissues

Collection of blood and tissues was performed under anesthesia ~18–22 h following the final heat test. Blood, taken from a carotid artery, was collected in a heparin-primed tube and immediately centrifuged to obtain plasma. Animals were then sacrificed and tissues samples removed, cleaned in ice-cold phosphate buffered saline, rapidly frozen using dry ice and stored at −80 °C. Tissue samples were homogenized and centrifuged to obtain the supernatants for analyses [10]. The remaining pellets were evaporated to dryness and weighed for data correction.

2.4. Blood and tissue assays

Blood and tissue assays were performed to analyze markers for inflammation (interleukins 1β and 6: IL-1β and IL-6), redox status (8-isoprostane, glutathione and superoxide dismutase or SOD) and stress (corticosterone and Hsp72). The following *Enzyme-Linked Immunosorbant Assay* (ELISA) kits sensitive to murine samples were used per manufacturers' instructions: plasma corticosterone (sensitivity: 30 pg/ml) and 8-isoprostane (sensitivity: 2 pg/ml) from Cayman Chemical (Ann Arbor, MI), plasma IL-1β (sensitivity: 4.8 pg/ml) and IL-6 (sensitivity: 1.8 pg/ml) from R&D Systems (Minneapolis, MN), and plasma glutathione (sensitivity: 10 μM) and tissue Hsp72 (sensitivity: 0.2 ng/ml) from Enzo Life Sciences (Plymouth Meeting, PA). Plasma and tissue homogenate supernatant samples were measured in duplicate. The aspirating and washing cycles were completed using an automatic microplate washer (Tecan Group Ltd., Switzerland). Samples were analyzed using the Magellan Data Analysis System (Tecan, Austria) and normalized to dry tissue weight (dw). Intra- and inter-assay coefficients of variation for ELISA concentrations were <10% for each assay.

Plasma SOD capacity was determined based on inhibition of superoxide anion produced by xanthine oxidase [12]. Lucigenin (5 μM)-enhanced chemiluminescence was used to assess changes in xanthine oxidase-dependent superoxide. The reaction was initiated by adding xanthine (100 μM) to phosphate-buffered saline (PBS) containing xanthine oxidase and plasma samples in a tube luminometer (Berthold AutoLumat Plus LB 953) at room temperature. The chemiluminescence signal was adjusted to background and continuously measured at 2 Hz. All measurements were performed in duplicate. Inhibition of superoxide was calculated as relative reductions in chemiluminescence and the plasma SOD activity was calculated from a standard curve of inhibition of superoxide by Cu/Zn-SOD (Sigma–Aldrich, St. Louis, MO). All plasma samples were run in the same assays to avoid inter-assay variability.

Hemolysis was assessed based on plasma lactate dehydrogenase (LDH) concentrations and colorimetric values. Plasma LDH was measured using an ELISA kit (sensitivity: 1.5 U/ml) from USCN Life Sciences Inc. (China). Direct absorbance at 414 nm of the plasma diluted in PBS was measured in a 96 well plate reader (Tecan Group Ltd., Switzerland) to quantitatively estimate free Hb content [13].

2.5. Blood hematocrit measurements

Packed red cell volume for each animal was determined by filling a micro-hematocrit capillary tube with blood, which was then sealed with a compound sealant. Hematocrit measurements were made with a CritSpin digital hematocrit reader following a centrifugation step with a CritSpin micro-hematocrit centrifuge per manufacturers' instructions (StatSpin, Norwood, MA).

2.6. Hemoglobin fractionation analysis

Each SCT mouse blood sample (10 μl) was subjected to the separation and quantitation of normal (HbA) and variant hemoglobin species (HbS) using a commercial HPLC resolution assay (Ultra^2 Resolution System, Trinity Biotech Plc., Ireland). The HPLC assays were performed by the Center for Sickle Cell Disease, Howard University Hospital (Washington, DC).

2.7. Data processing and statistical analysis

Data are expressed as mean ± SD. One-way ANOVA with Dunnett's post hoc tests and two-way ANOVA with Bonferroni's post hoc tests were used for comparison. The results were considered significant at $P \leq 0.05$.

3. Results

SCT mice were smaller in size than WT mice (SCT 22.1 ± 0.8 g versus WT 25.4 ± 0.4 g, $P < 0.001$). SCT and WT mice gained similar amounts of weight during the week between the two heat tests (SCT 1.2 ± 0.2 g versus 1.3 ± 0.3 g, $P > 0.05$). Four WT mice did not complete the first heat test due to a severe hyperthermic response (peak Tc > 42.4 °C) and were excluded from further tests. No significant differences were found in peak Tc or average La between SCT and WT mice during heat exposure (Table 1). There was no correlation between the measured HbS percentage and peak Tc values during heat exposure performed (Fig. 1). To examine how SCT and WT mice responded to similar thermal stress at local and systemic levels, we eliminated an additional 3 WT mice with a peak Tc greater than the highest value of SCT mice. Thus, SCT and WT mice selected for subsequent comparisons had a similar thermal response profile during heat exposure (Fig. 2.).

Table 1
Body core temperatures (Tc) and locomotor activity (La) of WT and SCT mice.

	Basal Tc (°C)	Heat test		
		Peak Tc (°C)	Peak Tc range (°C)	La (counts/min)
WT ($n = 18$)	36.2 ± 0.2	40.8 ± 0.6	40.2–42.0	5.4 ± 1.5
SCT ($n = 16$)	36.0 ± 0.2	40.8 ± 0.4	40.2–41.4	5.3 ± 2.1

Peak Tc values were calculated as the averages of Tc during the last 3 min of heat exposure.