LETTER TO THE EDITOR

Colchicine reduces inflammation in a humanized transgenic murine model of sickle cell disease

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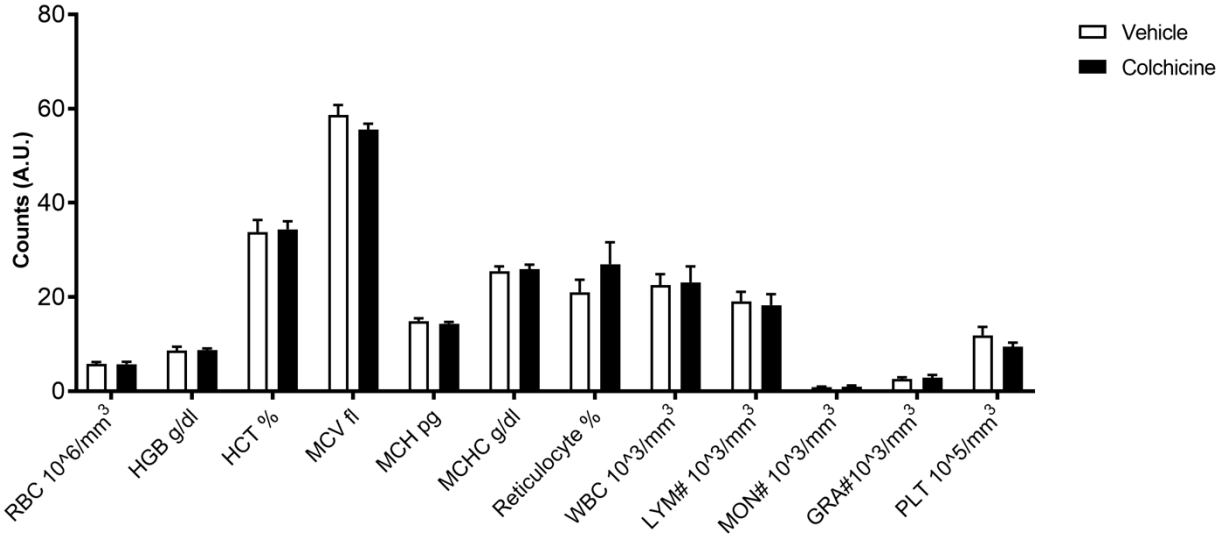
Supplement

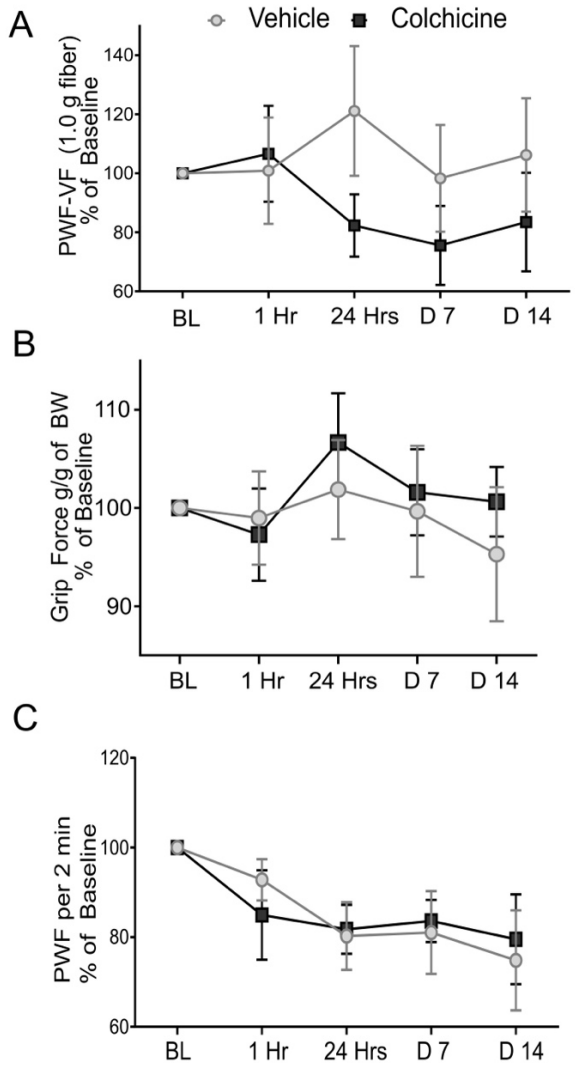
Supplement Figure 1



Supplement Figure 1. Colchicine did not alter circulating blood cell parameters.

Whole blood was combined with 100 mM EDTA pH 7.5 at a 2:1 ratio and assayed immediately for red blood cells (RBC), RBC indices, hemoglobin, platelets, and leukocytes with differential using an automated cytometer (VetPlus, Viernheim, Germany). Leukocyte differential was confirmed by review of Wright-stained (Wright Stain Solution, Sigma Aldrich, St Louis, MO, USA) peripheral blood smears. Equal volumes of blood and reticulocyte stain (Sigma Aldrich, St Louis, MO, USA) were mixed and incubated for 15 minutes at room temperature; microscopic slides were prepared for enumeration of RBC and reticulocytes (red cells containing purple inclusions or filaments). Equal volumes of blood and 2% sodium metabisulfate (Sigma Aldrich, St Louis, MO, USA) were gently mixed and incubated for 25 minutes at room temperature; then fixed with an equal volume of formalin buffer (Fisher Scientific, Hampton, NH, USA). A drop of the fixed blood was placed on a slide, covered with a cover slip, sealed with nail varnish, and allowed to stand at room temp for 1 to 4 hours. The slides were examined microscopically for enumeration of sickled and total RBC.

Supplement Figure 2



Supplement Figure 2. Colchicine did not alter hyperalgesia.

Hyperalgesia testing was performed at 1 hour and 24 h after the 1st dose of treatment and on days 7 and day 14. Hyperalgesia was analyzed after acclimatizing the mice in a quiet room at constant temperature for 30 mins. An interval of 15 min was maintained between testing for mechanical, deep tissue/musculoskeletal, and cold hyperalgesia. Data represent the mean and SEM from three trials each. (A) Mechanical hyperalgesia: Mice were placed into glass enclosures (10 × 6.5 × 6.5 cm) on an elevated wire mesh. A von Frey monofilament (Stoelting Co, Wood Dale, IL, USA) with 9.8 mN (1.0 g) calibrated bending force was applied to the mid-plantar surface of the hind paws and the paw withdrawal frequency (PWF) upon 10 applications was measured per hind paw. (B) Musculoskeletal/deep tissue hyperalgesia: Each mouse was held by its tail and allowed to grip the wires on a square grid. The peak tensile force exerted by the forelimb prior to grip release was measured using a computerized grip force meter (Columbus Instruments, OH, USA). The peak force exerted was recorded in grams and normalized by body weight. (C) Cold

hyperalgesia: Mice were gently placed onto a cold plate (Ugo Basile, Stoelting, Wood Dale, IL, USA, cat. no. 55100) maintained at ~4°C and the PWF during a 2 min period was recorded.