Key endothelial cell angiogenic mechanisms are stimulated by the circulating milieu in sickle cell disease and attenuated by hydroxyurea

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Supplementary Information

Patients – Diagnosis and Definitions of "steady-state" and Criteria for Commencing Hydroxyurea (HU) Therapy

HbSS and HbSC were diagnosed by hemoglobin electrophoresis and high-pressure liquid chromatography. Steady-state patients were afebrile and had not received blood transfusions nor experienced any vaso-occlusive episode during the previous 3 months. Patients on HU therapy had received 15–30 mg HU/kg/d for at least 12 months. Criteria for initiating HU therapy were, generally, frequent painful crises, acute chest syndrome, stroke and/or severe hemolytic anemia.

Plasma Preparation

Plasma was separated from EDTA blood by centrifugation (15 min, 2-8°C, 1 000xg) within 30 minutes of collection and subjected to an additional centrifugation step (10 min, 10 000xg, 2-8°C) before filtering through 0.2 µm-pore size filters (Millipore, Billerica, USA) to ensure complete platelet removal. Plasma was maintained at -80° C and repeated freeze-thaw cycles avoided.

Bioplex Assay

Two separate duplicate Bio-Plex runs were performed for each sample using the Bioplex 200 system (Bio-Rad Laboratories, Hercules, California, USA) at the Life Sciences Core Facility (LaCTAD), University of Campinas, Brazil. The calibration curve for each analyte was calculated with a 5-PL logistic regression curve (human bFGF utilized a 4-PL curve fit).

Animals

Berkeley SCD mice (Tg[Hu-miniLCRα1GγAγδβS] Hba–/–Hbb–/–),[1] originally purchased from the Jackson laboratories (Bar Harbor, ME), and C57BL/6 mice were maintained and bred at the animal housing facility (CEMIB), University of Campinas, Brazil. Chimeric male SCD mice (hereafter denominated "SCD mice") were generated by transplantation of nucleated bone marrow cells harvested from Berkeley mice into lethally-irradiated C57BL/6 mice (2-months old), as previously described.[2] Only chimeric SCD mice expressing > 97% human globin were utilized in experiments at 3 months after transplantation. Chimeric male C57BL/6/ C57BL/6 mice (denominated "non-SCD mice") were also generated for some experiments by transplanting C57BL/6 marrow cells into lethally-irradiated C57BL/6 mice (2-months old) and were utilized in experiments at 3 months after transplantation. Animals were fed on a 22% protein diet (NUVILAB – CR1 irradiated) for at least 3 months before experiments.

Statistical Analysis

One-way analysis of variance followed by Holm Sidak's post test and the Kruskall Wallis test with Dunn's posttest were used for comparing parametric and non-parametric data, respectively. Differences between experimental groups were considered as statistically significant when $P<0.05$.

Supplementary References

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