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No evidence for cell activation or brain vaso-occlusion with plerixafor mobilization in sickle cell mice

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ABSTRACT

Gene therapy for sickle cell disease is currently in active trials. Collecting hematopoietic progenitor cells safely and effectively is challenging, however, because granulocyte colony stimulating factor, the drug used most commonly for mobilization, can cause life-threatening vaso-occlusion in patients with sickle cell disease, and bone marrow harvest requires general anesthesia and multiple hip bone punctures. Plerixafor is an inhibitor of the CXCR4 chemokine receptor on hematopoietic progenitor cells, blocking its binding to SDF-1 (CXCL12) on bone marrow stroma. In support of a clinical trial in patients with sickle cell disease of plerixafor mobilization (NCT02193191), we administered plerixafor to sickle cell mice and found that it mobilizes hematopoietic progenitor cells without evidence of concomitant cell activation or brain vaso-occlusion.

1. Introduction

Sickle cell disease (SCD) is caused by a single nucleotide base change in the β-globin gene and is thus an excellent candidate for gene therapy. In fact, gene therapy for SCD is currently in active trials, but collection of hematopoietic progenitor cells (HPCs) safely and effectively remains a challenge. Granulocyte colony stimulating factor (G-CSF), the drug used most commonly for collecting HPC, can cause life-threatening vaso-occlusion in SCD, including multi-organ failure [1]. Bone marrow harvest requires general anesthesia and multiple hip bone punctures. Plerixafor is an inhibitor of the CXCR4 chemokine receptor on HPC, interfering with its binding to SDF-1 (CXCL12) on bone marrow stroma. Plerixafor alone, without concomitant G-CSF, may have excellent mobilization efficacy in SCD patients, as demonstrated by a clinical trial showing safety and efficacy of mobilization with plerixafor alone was superior to G-CSF in splenectomized β-thalassemia patients [2]. As pre-clinical data in support of a clinical trial in SCD patients studying plerixafor mobilization (NCT02193191), we administered plerixafor to SCD mice to assess HPC mobilization; platelet, endothelial, and neutrophil activation; and brain vaso-occlusion.

2. Materials and methods

2.1. Sickle mice

All mouse experiments were approved by the NYBC and Einstein Institutional Animal Care and Use Committee and performed between July 2014 and February 2015. Male and female 3–6 month old SS Berkeley (stock number 003342, The Jackson Laboratory, Farmington, CT) or SS Townes mice (stock number 013071, The Jackson Laboratory, Farmington, CT) were used for all experiments.

A cohort of Berkeley mice at Einstein (n = 8: 4 plerixafor, 2 G-CSF, 2 saline) was used for the initial 4 experiments performed, where cerebral blood flow was assessed by MRI prior to sacrifice for mobilized peripheral blood and HPC assessments. A G-CSF (positive control) or saline (negative control) was included with each plerixafor-treated mouse. The two subsequent experiments (n = 9 per experiment: 3 plerixafor, 3 G-CSF, 3 saline) were performed with Townes sickle mice at New York Blood Center, and only mobilized peripheral blood and HPC assessments were performed.

2.2. Treatment protocol

Mice were randomized to either subcutaneous treatment with plerixafor (Mozobil, Genzyme-Sanofi) 10 mg/kg once; G-CSF (Neupogen, Amgen, Thousand Oaks, CA) 250 μg/kg daily for 5 days as the positive control, or equivalent volume (5 μL/g) normal saline once

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or daily for 5 days as the negative controls for plerixafor and G-CSF, respectively. Since peak plerixafor mobilization in mice occurs at 1 h [3], peripheral blood was harvested into EDTA by cardiac puncture at 1–2 h post-dose in plerixafor-treated mice and their saline controls; blood from G-CSF-treated mice and their saline controls was harvested shortly (3–5 h) after the 5th dose, the peak of mobilization in mice for G-CSF [4]. Platelet-poor plasma was made immediately, and the rest of the blood was transferred into Microtainer tubes (BD, Franklin Lakes, NJ) and stored on ice until CBC and flow cytometry analysis.

2.3. Cerebral vaso-occlusion assessment

The Berkeley mice underwent brain imaging on a 9.4 Tesla MR/MRS system (Agilent Inc., Santa Clara, CA) pre-treatment and then after treatment (before euthanization). Imaging included brain perfusion assessment using FAIR arterial spin labeling, Diffusion Tensor Imaging (DTI) and T2-weighted imaging. Image data were registered to the Paxinos-Franklin mouse atlas [5], and reduced to 6 regions defined as cortex (COR = FRO, MOT, SOM, AUD, VIS, CTXG), white matter region (WM = CC + EC + AC), hippocampus (HCP = PERI, ENT, CA1, CA3, DG and HIPG), basal ganglia (BG = COLLIC, PIT, HY, IIN, CP, BGG, FXS, INT, CPED), substantia nigra (SN), and thalamus (THAL = TH, AMY, MBG). Regional image-based assessment of brain tissue perfusion before and after treatment allows assessment of changes in tissue microcirculation that take place if cerebral vaso-occlusion develops [6]. DTI mean diffusivity (MD) is a sensitive marker of cerebral water diffusion and exchange and DTI fractional anisotropy (FA) is a sensitive measure of axonal integrity and myelin density. Acute ischemia is detected with DTI-MD, with cerebral vascular occlusion typically leading to FA changes, which reflect alterations in myelin water content or cytoskeletal changes resulting from ischemic axonal damage. Larger or more severe strokes typically lead to increased tissue water content as tissue permeability change ensues, and is detected by T2-weighted MRI, a sensitive marker of inflammation and edema.

2.4. CBC and WBC differentials

Whole blood samples undiluted or diluted 1.25–5 times with PBS were analyzed with the Advia 120 Hematology System (Siemens, Malvern, PA).

Flow Cytometry Analysis for Hematopoietic Progenitor Cells and Neutrophil Activation. Whole blood samples were red cell lysed and analyzed using the BD LSRII system with FACS software (BD, Franklin Lakes, NJ).

For enumeration of HSC mobilization (Lin-Sca-1$^+$c-kit$^+$Flt3$^-$ or LSKF cells) [7], cells were stained with Mouse Lineage Panel (BD, Franklin Lakes, NJ), streptavidin PE (eBioscience, San Diego, CA), anti-mouse Sca-1 PE-Cy7 (eBioscience, San Diego, CA), anti-mouse c-kit FITC (eBioscience, San Diego, CA), anti-Flt3 APC (eBioscience, San Diego, CA), and 7-ADD (BD, Franklin Lakes, NJ).

For evaluation of neutrophil L-selectin shedding, cells were stained with anti-mouse Gr-1 FITC (eBioscience, San Diego, CA), anti-mouse CD115 PE (eBioscience, San Diego, CA), anti-mouse CD62L APC (eBioscience, San Diego, CA), and 7-AAD (BD, Franklin Lakes, NJ).

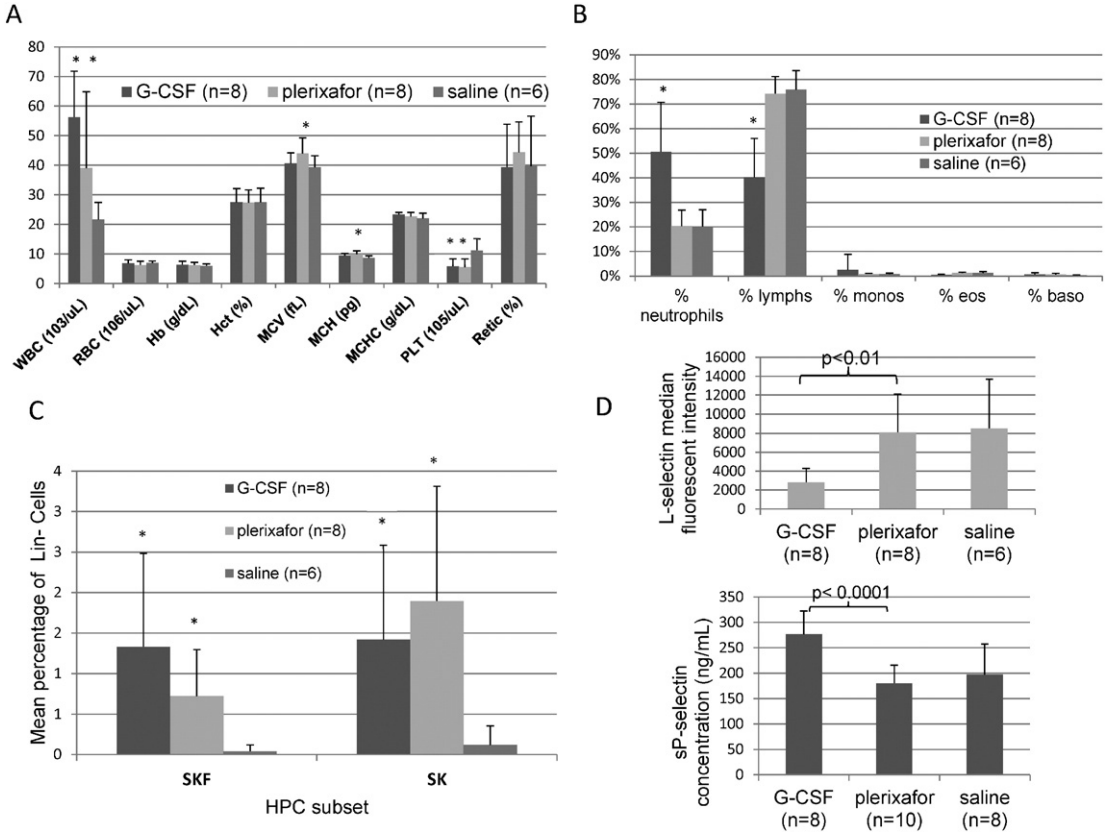

Fig. 1. Comparison of mobilization parameters with plerixafor, G-CSF, and saline. A. Complete blood count parameters. White blood cell and platelet counts of both plerixafor and G-CSF treated mice were significantly different from saline control mice (*$p < 0.02$). B. White blood cell differentials. Neutrophil and lymphocyte percentages of G-CSF treated mice were significantly different from saline control mice (*$p < 0.005$). C. Hematopoietic progenitor cell (HPC) subsets. Percentages of Sca-1$^+$c-kit$^+$Flt3$^+$ (SKF) and Sca-1$^+$c-kit$^+$ (SK) HPC in the lineage-negative population were significantly higher in both plerixafor and G-CSF treated mice compared to saline (*$p < .02$). D. Cell activation markers neutrophil L-selectin (CD62L, decreased with activation) and soluble plasma P-selectin (increased with endothelial/platelet activation). Cell activation markers were significantly decreased with plerixafor compared to G-CSF.