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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.

Keywords

Plerixafor; Sickle cell disease; Hematopoietic progenitor cell mobilization; Platelet activation; Neutrophil activation; Endothelial cell activation

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Authorship contributions and disclosure of conflicts of Interest

EC: conducted the study and analyzed data.

CB: designed the study, analyzed data, and wrote the manuscript.

MC: conducted the mouse imaging studies.

KY: analyzed data and reviewed the manuscript.

MN: designed the study and reviewed the manuscript.

HB: designed the study and reviewed the manuscript.

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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. Sickie 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. AG-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 μ g/kg daily for 5 days as the positive control, or equivalent volume (5 μ L/g) normal saline once 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).

2.5. Platelet/endothelial activation assessment

Within 30 min of collection, whole blood was centrifuged at 1000 g at 4 °C for 15 min and 4600 g at 4 °C for 10 min to obtain platelet-poor plasma. Plasma was stored at –20 °C until analysis. Soluble P-selectin was measured by ELISA following the manufacturer protocol (R&D systems, Minneapolis, MN) for evaluation of platelet/endothelial activation.

2.6. Statistical analysis

Groups were tested for normality and then compared using the appropriate t-test (Mann–Whitney U for non-parametric data). Significance was assessed at the $p < 0.05$ level. Figures show mean $\pm$ standard deviation and significant p-values compared to saline when not otherwise indicated.

Data was pooled for the two mouse models: all data trended in the same direction except for the LSK analysis; the data for each mouse model can be found in Supplementary Data (Table 1). Flow cytometry-based assays were not evaluable for 4 Berkeley mice (2 plerixafor-treated and the 2 saline-treated) due to poor cell viability ($<25\%$ by 7-AAD) with the time interval between blood draw and analysis, and CBC data for these mice was therefore also excluded from analysis. The mean viabilities of all other samples were $78 \pm 11\%$ and $67 \pm 13\%$ for the HPC and neutrophil analyses, respectively.

3. Results and discussion

Plerixafor and G-CSF were absorbed as evidenced by expected changes in WBC and platelet counts with treatment compared to saline. CBC showed the mean WBC and platelet counts of both plerixafor and G-CSF groups to be significantly different from saline (Fig. 1A), but the WBC differential was significantly different (in % and absolute numbers of neutrophils and lymphocytes) from saline only in the G-CSF group (Fig. 1B).

Both plerixafor and G-CSF significantly mobilized HPC subsets. The percentages of HPC subsets were significantly higher in both plerixafor and G-CSF groups compared to saline, with no significant differences between plerixafor and G-CSF (Fig. 1C). The standard deviations are high in the HPC subsets of the G-CSF and plerixafor-treated mice, which may be related to the fact that mice from several litters were distributed between the treatment groups. Although this study showed a trend towards higher mobilization with G-CSF of the more primitive LSKF subset, prior published data suggest that plerixafor alone mobilizes a higher number of more primitive HPCs than G-CSF alone [2,8]. Furthermore, mobilization with plerixafor may have been suboptimal due to splenic enlargement in sickle mice, given that splenectomy may increase the efficacy of plerixafor mobilization [2,9].

In support of potential safety of plerixafor in SCD patients, there was no evidence of neutrophil or platelet/endothelial activation with plerixafor. Following neutrophil activation, L-selectin is shed by proteolytic cleavage [10]. P-selectin is expressed on both platelets and endothelial cells and soluble P-selectin is a marker of their activation [11]. Neutrophil L-selectin was significantly lower and soluble P-selectin significantly higher with G-CSF compared to either plerixafor or saline (Fig. 1D), suggesting that there is less neutrophil activation with plerixafor. Blood for these studies was drawn at the time reported for peak HPC mobilization in mice for the respective drug [3,4]. It is possible that, with plerixafor, cell activation does not occur coincident with HPC mobilization and thus detection of cell activation was missed. However, with G-CSF, platelet/endothelial [12] and neutrophil activation [13] occur coincident with HPC mobilization, and in the one paper of which we are aware that reported on cell activation with plerixafor (in multiple myeloma patients also

given G-CSF), 4 h after plerixafor administration no increased adhesion marker expression on CD34+ cells was observed [14].

Berkeley mice show evidence of ischemic brain injury at baseline [15]. MRI brain imaging and analysis post- versus pre-treatment, however, revealed no significant changes (paired t-tests, $p > 0.37$) in any treatment group, including the G-CSF group that had clear evidence of cell activation; possibly cell activation was not high enough to cause vaso-occlusion. Interestingly, however, there was a trend towards decreased cerebral blood flow after plerixafor treatment (Fig. 2A) without any increases in DTI MD (Fig. 2B), which measures tissue water diffusivity, or DTI FA, which measures axonal and myelin integrity. Vaso-occlusion causing tissue hypoxia would have resulted in a shift of extracellular water to the intracellular compartment that should have been detected by the DTI-MD and FAIR measurements. In tandem with the lack of cell activation with plerixafor, one may hypothesize that decreased CBF (if the trend is real) does not necessarily reflect vaso-occlusion. Sick cell patients, for example, have an acute decrease in CBF with red cell transfusion that is unrelated to vaso-occlusion [16]. Berkeley sickle mice have a very high cerebral blood flow (CBF) at baseline which is only partly related to the degree of anemia (Fig. 2C, C. Branch, personal communication). If the plerixafor decreased blood pressure, possibly as a known reported side effect of plerixafor or from decreased systemic vaso-occlusion resulting in increased perfused volume and thus transiently decreased blood pressure, this would be reflected by a decreased CBF not reflective of vaso-occlusion.

In conclusion, our pre-clinical data in sickle cell mice suggest that plerixafor may be safe and effective for HPC mobilization in SCD patients, and support continuation of a clinical trial in SCD patients studying plerixafor mobilization (NCT02193191) for gene therapy. Use of plerixafor alone in patients with SCD may allow for HPC mobilization for curative gene therapy without the vaso-occlusive side effects seen with G-CSF.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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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.

Fig. 2.

MRI studies in plerixafor-treated mice (n = 4). A. Cerebral blood flow (CBF). There was no significant change in CBF. B. DTI-mean diffusivity. There was no significant change in mean diffusivity. C. MRI images. Berkeley SS mice exhibit high CBF at baseline. Left: T2-weighted anatomic image pre-plerixafor. Middle: Cerebral perfusion image pre-plerixafor. Right: Cerebral perfusion image post-plerixafor. The scale to the right is CBF, in mL/100 g/min. Abbreviations: WM = white matter, COR = cortex, HCP = hippocampus, BG = basal ganglia, SN = substantia nigra, THAL=thalamus.