Brief report

BIO5192, a small molecule inhibitor of VLA-4, mobilizes hematopoietic stem and progenitor cells

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Here we show that interruption of the VCAM-1/VLA-4 axis with a small molecule inhibitor of VLA-4, BIO5192, results in a 30-fold increase in mobilization of murine hematopoietic stem and progenitors (HSPCs) over basal levels. An additive effect on HSPC mobilization (3-fold) was observed when plerixafor (AMD3100), a small molecule inhibitor of the CXCR-4/SDF-1 axis, was combined with BIOS5192. Furthermore, the combination of granulocyte colony-stimulating factor (G-CSF), BIO5192, and plerixafor enhanced mobilization by 17-fold compared with G-CSF alone. HSPCs mobilized by BIO5192 or the combination of BIO5192 and plerixafor mobilized long-term repopulating cells, which successfully engraft and expand in a multilineage fashion in secondary transplantation recipients. Splenectomy resulted in a dramatic enhancement of G-CSF-induced mobilization while decreasing both plerixafor- and BIO5192-induced mobilization of HSPCs. These data provide evidence for the utility of small molecule inhibitors of VLA-4 either alone or in combination with G-CSF or AMD3100 for mobilization of hematopoietic stem and progenitor cells. (Blood. 2009;114:1340-1343)

Introduction

Hematopoietic stem and progenitor cells (HSPCs) reside within a specialized microenvironment referred to as the stem cell niche, which regulates critical HSPC processes, such as self-renewal and differentiation.1-3 Although different anatomic niches have been described during ontogenesis, hematopoiesis in the adult mouse is generally restricted to the spleen and bone marrow.4 Trafficking of HSPCs between the bone marrow, peripheral blood, and secondary organs is a dynamic process. In response to physiologic stressors or exogenous administration of cytotoxic agents, cytokines, and chemokines, HSPCs can mobilize into the peripheral circulation. Conversely, after infusion into lethally irradiated mice, HSPCs are able to home and engraft in the marrow and spleen to restore normal hematopoiesis. HSPC mobilization and homing are thought to be closely related processes centered around 2 critical pathways: one involving the α4β1 integrin, VLA-4, with its ligand VCAM-1, and the other chemokine receptor CXCR4 and its ligand SDF-1.

In this paper, we present data with a small molecule inhibitor of VLA-4, BIO5192, and its effects on mobilization of HSPCs. Furthermore, we examine the combination of BIO5192 with plerixafor, a CXCR4 antagonist, to characterize the ability of these compounds, alone, or in combination with granulocyte colony-stimulating factor (G-CSF) to mobilize HSPCs from different anatomic niches.

Methods

Mice and reagents

Mouse strains 129Sv/J, C57BL/6J, and B6.SJL-Ptprca Pepcb/H9251 were obtained from The Jackson Laboratory. Plerixafor (Genzyme Corporation) and rHu-G-CSF (Amgen) were supplied as sterile isotonic aqueous solutions. BIO5192 was supplied by AnorMED as a sterile powder and reconstituted at 200 μg/mL in pH 7.0 ethanol:propylene glycol:water (10:36:54). Animal care and euthanasia protocols were approved by the Animal Studies Committee of Washington University School of Medicine.

Binding assays

Cell adhesion assays to fibronectin-coated plates and binding assays to human VCAM-1/Fc chimera protein (R&D Systems) were performed per the manufacturer’s directions as described.5 Murine A20 B-cell and human Jurkat T-cell leukemia cell lines were obtained from ATCC.

Progenitor analysis and transplantation

HSPC assays were performed in methylcellulose-containing Iscove modified Dulbecco medium supplemented with interleukin-3, interleukin-6, and stem cell factor (MethoCult3534; StemCell Technologies).6 For competitive stem cell repopulation transplantation experiments, peripheral blood monocellular cells (PBMCs) from wild-type C57BL/6 (CD45.2+) mice mobilized by G-CSF, plerixafor, BIO5192, or the combination were mixed with 0.5 × 10⁶ competitor bone marrow from wild-type mice congenic at the CD45.1+ locus (B6.SJL-Ptprca Pepe/H11003; BoyJ) and transplanted into lethally irradiated (900 cGy) C57BL/6 x B6.SJL-Ptprca Pepe/H11001; BoyJ F1 (CD45.1+/CD45.2+) recipients. The entire blood volumes of 3 donor mice (3.0-3.5 mL total volume) were pooled for each recipient. Secondary transplantation were performed by injecting 10⁶ unfractionated bone marrow cells from donors 5 months after transplantation intravenously into lethally irradiated (900 cGy) secondary recipients (CD45.1+CD45.2+ compound heterozygotes).

Statistical analysis

Analyses were performed using analysis of variance on log-transformed colony-forming unit (CFU) values, adjusting for within-subject correlation.
Figure 1. Mobilization of hematopoietic stem and progenitor cells by BIO5192. (A) Calcein-AM labeled A20 cells were seeded in bovine serum albumin (BSA)-coated or fibronectin-coated plates and treated with phorbol 12-myristate 13-acetate and/or BIO5192 1 μg/mL. Cell adhesion is expressed as the percentage of fluorescence after removal of unbound cells compared with fluorescence of the total applied cells. (B) A20 cells incubated with recombinant human VCAM-1/Fc chimera protein plus or minus BIO5192 1 μg/mL for 30 minutes. Binding of VCAM-1 was detected using phycoerythrin-donkey anti–human Fc analyzed by fluorescence-activated cell sorter and compared with a phycoerythrin-conjugated donkey IgG (isotype control). (C-E) Colony-forming cell assays. C57BL/6J x 129Sv/J F1 mice were analyzed for peripheral blood CFU-GM after treatment with (C) plerixafor at 1, 3, or 5 mg/kg subcutaneously or intravenously. (D) BIO5192 at 0.001, 0.01, 0.1, 1, or 3 mg/kg intravenously. (E) Plerixafor 5 mg/kg subcutaneously and BIO5192 1 mg/kg intravenously alone or in combination or (F) G-CSF 250 μg/kg per day 5 days alone, in combination with plerixafor 5 mg/kg subcutaneously, or BIO5192 1 mg/kg intravenously or the 3-drug combination. (G) Competitive repopulation assay. Lethally irradiated CD45.1/CD45.2 mice received transplants of 0.5 x 10^6 congenic CD45.1 bone marrow competitor cells plus PBMCs from untreated mice or those mobilized with G-CSF 5 mg/kg per day of G-CSF × 5 days, BIO5192 1 mg/kg intravenously, plerixafor 5 mg/kg subcutaneously, or the combination of BIO5192 1 mg/kg intravenously and plerixafor 5 mg/kg subcutaneously (n = 3 mice/group). Peripheral blood was harvested on day 5 for G-CSF–treated mice, 1 hour after injection for BIO5192, and 3 hours after injection for plerixafor and plerixafor + BIO5192–treated mice. The contribution of mobilized cell populations to hematopoiesis was determined by flow cytometry for CD45.2+ donor cells. (H) Secondary transplantation. A total of 10^6 bone marrow cells from primary transplant recipients were injected into lethally irradiated C57BL/6 secondary mice (CD45.1+/CD45.2−; n = 3 mice/group). Engraftment was assessed monthly for 3 months by peripheral blood flow cytometry for CD45.2+ donor cells. Data are mean ± SEM. *P < .05; **P < .01; ***P < .001.
Results and discussion

HSPC mobilization by BIO5192

BIO5192 is a selective and potent small molecule inhibitor of VLA-4, with an affinity of 250- to 1000-fold higher than for the related αβ7 integrin. To confirm the activity of BIO5192, we assessed the binding of VLA-4 expressing murine A20 lymphoma cell line (ATCC) to fibronectin-coated dishes and a soluble VCAM-1/Fc fusion. BIO5192 reduced both untreated and phorbol 12-myristate 13-acetate–stimulated cell binding to fibronectin-coated plates by 43% and 36%, respectively, indicating that BIO5192 blocks binding to multiple activation states of VLA-4 (Figure 1A; P < .001). Likewise, BIO5192 inhibited binding of soluble VCAM-1 (Figure 1B). Similar results were obtained with human Jurkat cells (supplemental Figure 1, available on the Blood website; see the Supplemental Materials link at the top of the online article).

To characterize the ability of BIO5192 to mobilize HSPCs, we treated mice with BIO5192 and plerixafor and assayed for mobilization of peripheral blood CFU-granulocyte macrophage (GM). Analysis of the dose-response relationship indicates that plerixafor intravenously resulted in more rapid mobilization (peak 1 hour) than subcutaneous administration (peak 3 hours; Figure 1C). Plerixafor doses higher than 3 mg/kg intravenously were lethal to the mice. In comparison, BIO5192 was more potent when administered intravenously over subcutaneously but mobilized with similar kinetics with an approximate 30-fold mobilization peaking at 0.5 to 1 hour (1500 CFU/mL compared with baseline of 50-120 CFU/mL; Figure 1D and supplemental Figure 2). Treatment using the BIO5192 diluent-only control did not demonstrate any change in CFU numbers over baseline (data not shown). The combination of plerixafor and BIO5192 exerted an additive effect on progenitor mobilization, which peaked at 3 hours and persisted for at least 6 hours (Figure 1E; P < .001 for plerixafor + BIO5192 compared with each agent alone at 3-8 hours). A similar, additive effect on HSPC mobilization was observed when the triplet of plerixafor, BIO5192, and G-CSF was tested (Figure 1F; P < .001 for G-CSF + plerixafor + BIO5192 compared with other treatments) with HSPCs increasing 135-fold over baseline.

Stem cell function of BIO5192-mobilized peripheral blood was also assessed in competitive long-term repopulating assays. Engraftment of mice that received transplants of HSPCs mobilized after a single dose of plerixafor and BIO5192 approached that of a 4-day course of G-CSF (Figure 1G). Higher levels of donor chimerism were observed for the combination of plerixafor and BIO5192 versus plerixafor (P < .002) or BIO5192 alone (P < .003). Secondary transplantation demonstrated stable engraftment of BIO5192-mobilized cells, suggesting that BIO5192, like plerixafor and G-CSF, mobilizes HSPCs, which provide long-term multilineage engraftment (Figure 1H). Although a trend toward reduced engraftment in the plerixafor arm at 3 months was observed, this difference was not statistically significant.

HPC mobilization in splenectomized mice

We then sought to assess the role of the spleen in BIO5192, plerixafor, and G-CSF–induced HSPC mobilization. Splenectomized mice had slightly higher numbers of circulating CFUs in the peripheral blood before treatment (mean CFU, 330/mL vs 120/mL, P = .02). Mobilization of splenectomized mice with 5 days of G-CSF resulted in an 87-fold increase in CFU/mL, an approximately 5-fold greater mobilization than wild-type mice treated with G-CSF (Figure 2). Mobilization with both plerixafor and BIO5192 alone was reduced in splenectomized mice. With the combination of plerixafor and BIO5192, the magnitude of HSPC mobilization in splenectomized mice decreased 4-fold (Figure 2B; P < .001).

Antibodies against VLA-4 and VCAM-1 have been shown to mobilize HSPCs and exert an additive effect when combined with G-CSF. In contrast to antibodies that exert their peak effect over several days, the use of small molecule inhibitors, such as BIO5192 and plerixafor, provides a more rapid and reversible mobilization with a peak mobilization occurring within minutes to hours of treatment. Here, we demonstrate the clinical potential of combining small molecule inhibitors of CXCR4 and VLA-4 as a rapid HSPC mobilization regimen. The development of a G-CSF–free mobilization regimen is attractive both to avoid potential toxicities of G-CSF and to save time and resources during a 4- to 5-day G-CSF–based mobilization.

Previous studies have shown that homing of HSPCs to the marrow but not the spleen was blocked by pretreatment of donor cells with...
antibodies to VLA-4.\textsuperscript{16} Both AMD3100 and BIO5192 mobilization of progenitors was reduced by splenectomy, indicating that both CXCR4 and VLA-4 are important for mobilization from both marrow and splenic niches. Whereas both G-CSF and plerixafor mobilize HSPCs by disrupting the SDF-1/CXCR4 axis, the effects of splenectomy are somewhat paradoxical. Although splenectomy augmented mobilization in response of G-CSF, it significantly reduced the number of circulating progenitors after plerixafor treatment. A similar difference was seen after BIO5192 treatment. We speculate that the spleen may serve as a reservoir to remove marrow-derived progenitors from the peripheral circulation but that the effect may be blunted by the G-CSF–induced proliferation of progenitors. Although this effect may not be as pronounced in human subjects where the spleen is less prominent in adult hematopoiesis, further studies are required to investigate these options.

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Authorship

Conflict-of-interest disclosure: J.F.D. and G.L.U. have received honoraria from Genzyme Corp. The remaining authors declare no competing financial interests.

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