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Targeted antagonism of CXCR4 mobilizes progenitor cells under investigation for cardiovascular disease

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Abstract

Background aims—Bone marrow (BM)-derived cells may repair cardiovascular injury but populations of interest circulate in small numbers. Cytokines such as granulocyte-colony-stimulating factor mobilize cells under investigation for this purpose, including CD133⁺ but require injections over multiple days and may promote inflammation. The purpose of this study was to evaluate the effects of a novel CXCR4 inhibitor (plerixafor), previously shown to mobilize CD34⁺ stem cells, on CD133⁺ mobilization and markers of inflammation.

Methods—Healthy subjects received a single subcutaneous injection of plerixafor in escalating doses: 240 mcg/kg (n = 3), 320 mcg/kg (n = 5) and 400 mcg/kg (n = 7). CD133⁺ and CD133⁺/VEGFR-2⁺ cells were measured by flow cytometry at baseline, then 4–6 h following plerixafor injection. Markers of inflammation in serum were measured at baseline, then again 10 h following injection of the 400 mcg/kg dose.

Results—Across all doses, white blood cells increased on average three-fold from baseline values. CD133⁺ cells increased on average 24-fold (from 616±141 cells/mL to 14 713±4423 cells/mL, P=0.0064) without clear evidence of a dose effect. CD133⁺/VEGFR-2⁺ cells ranged from 0 to 20 cells/mL at baseline and from 0 to 124 cells/mL following plerixafor administration, although the rarity of these cells precluded a statistical analysis of this population. C-reactive protein and

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serum amyloid type A were not increased after the 400 mcg/kg dose. Pro-inflammatory cytokine levels were undetectable before and after plerixafor, except for macrophage inflammatory protein-1 beta, which increased slightly but significantly after the 400 mcg/kg dose of plerixafor ($P=0.0156$).

Conclusions—CD133⁺ cells are mobilized into the circulation following a single injection of the CXCR4 antagonist plerixafor, without clear evidence for systemic activation of inflammation. This effect may be of importance in cell-based approaches for treating cardiovascular diseases.

Keywords

Cardiovascular disease; CXCR4 antagonism; heart disease; inflammation; progenitor cells; vascular repair

Introduction

There is considerable interest in bone marrow (BM)-derived cell-based approaches to cardiovascular diseases, with more than 200 clinical trials filed in ClinicalTrials.gov, accessed as on August 3, 2009 that utilize direct infusion or injection of cells into hearts of patients with myocardial infarction, severe angina or heart failure. A progenitor population of interest is defined by CD133⁺ and CD133⁺/VEGFR-2⁺ cell-surface expression [1,2], which circulate in small numbers even in healthy individuals (c. 0.002% of total monocytes in the peripheral blood) [3]. Accordingly, collection of these, and other BM-derived cells of interest for the treatment of cardiovascular diseases, in sufficient quantities for cell-based approaches requires either aspiration from BM or cytokine mobilization. Concerns have been raised, however, about pro-inflammatory effects of granulocyte-colony-stimulating factor (G-CSF) administration, currently used for hematopoietic stem cell mobilization, with reports of accelerated angina, myocardial infarction and death [4–10].

Recently, a novel CXCR4 antagonist (plerixafor) has been shown to mobilize hematopoietic progenitor cells into the circulation after a single injection [11,12]. Plerixafor has also been demonstrated to have anti-inflammatory effects [13], a property that could be important for patients with atherosclerotic cardiovascular disease if considered for progenitor cell mobilization. Our purpose was to study the effect of a single injection of plerixafor, administered in escalating doses to healthy volunteers, on mobilization from BM of CD133⁺ and CD133⁺/VEGFR-2⁺ cells, under investigation for cardiovascular disease, and serum markers of inflammation.

Methods

Following informed consent for participation in a protocol approved by the institutional review board of the National Heart, Lung and Blood Institute (Bethesda, MD, USA), plerixafor was given as single subcutaneous injections in escalating doses (240 mcg/kg, 320 mcg/kg and 400 mcg/kg) to 10 healthy subjects (six males and four females; average age 31±3 years), with several receiving a second injection at a higher dose 2 or more weeks following the initial dose.

Flow cytometry

White blood cells and absolute numbers of granulocytes and lymphocytes were measured before and 6 h after injection. Flow cytometric analysis was performed on approximately 10⁶ mononuclear cells per sample to quantify CD133⁺ and CD133⁺/VEGFR-2⁺ cells using fluorochrome-conjugated antibodies CD133–allophycocyanin (APC) (Miltenyi Biotec, Auburn, CA, USA) and VEGFR-2–phycoerythrin (PE) (R&D Systems, Minneapolis, MN,

USA). Lymphocytes of B- and T-lineages were excluded from analysis using fluorochrome-conjugated antibodies with appropriate isotype controls, CD3–fluorescein isothiocyanate (FITC) and CD19–FITC (BD Biosciences, San Jose, CA, USA); immature granulocytes were removed with CD33–FITC. All cells were stained with either 7AAD (BD Biosciences) or Live/Dead (BD Biosciences) to allow exclusion of dead cells. A subset of samples was stained with Hoescht (Invitrogen, Carlsbad, CA, USA) to ensure the flow cytometer was counting nucleated cells (<1% of cells were non-nucleated). The Cyan flow cytometer was used with Summit Software (Dako, Ft Collins, CO, USA) for data acquisition and FCSExpress (De Novo Software, Thornhill, Canada) for analysis.

Assays for markers of inflammation

Markers of inflammation were measured in the serum from seven subjects at baseline and 10 h after 400 mcg/kg plerixafor. Levels of C-reactive protein were measured using a high-sensitivity (0.1 mg/L), solid-phase, chemiluminescent immunometric assay (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). The interassay coefficients of variation (CV) were <10% for high sensitivity C-reactive protein levels between 1.3 and 94.0 mg/L. Cytokine profiles, including a panel of pro-inflammatory cytokines (interferon-gamma, tumor necrosis factor-alpha, interleukin-6 and macrophage inflammatory protein-1 beta) and serum amyloid A, were measured using a multiplex assay (R&D Systems).

Statistical analysis

Data are given as mean±SEM. Comparisons of responses to plerixafor injections were performed using the Student's *t*-test for paired samples when data were normally distributed and the Wilcoxon signed-rank test for skewed data.

Results

Across all dose levels, white blood cells, granulocytes and lymphocytes at 6 h post-plerixafor administration increased on average three-fold from baseline values (Table I). CD133⁺ cells increased on average 24-fold to 14 713±4423 cells/mL (*P*=0.0064) without a clear dose-effect on mobilization (Figure 1). CD133⁺/VEGFR-2⁺ cells were rare events, ranging from 0 to 20 cells/mL at baseline and 0 to 124 cells/mL following plerixafor administration. Because of the rarity of these cells, statistical analysis was not performed on this population.

At 10 h following administration of plerixafor 400 mcg/kg, there was no change in levels of high sensitivity C-reactive protein (1.1±0.6 mg/L baseline versus 0.9±0.4 mg/L post-plerixafor, *P*=0.9375) or serum amyloid type A levels (11.0±3.8 mg/L baseline versus 11.1±3.3 mg/L post-plerixafor, *P*=0.5781). All cytokines tested were beneath the lower limits of detectability at baseline and at 10 h post-treatment, with the exception of macrophage inflammation protein-1 beta, which increased slightly but significantly (from 41±3.8 pg/mL to 67.6±10.9 pg/mL, *P*=0.0156).

Discussion

The CXCR4 antagonist plerixafor is approved in the USA (Mozobil) in combination with G-CSF for hematopoietic stem cell mobilization and collection for subsequent autologous transplantation in non-Hodgkin's lymphoma and multiple myeloma patients. Plerixafor inhibits stromal-derived factor-1 binding to CXCR4 within BM, rapidly mobilizing hematopoietic stem cells into the circulation [11,12]. Our data show that CD133⁺ cells, currently under investigation for heart treatment in at least four clinical trials, are mobilized rapidly into the circulation following a single injection of plerixafor. The CD133⁺ subtype

with VEGF receptor expression (VEGFR-2), reported to be important in vasculogenesis [1,2], was rare before and following plerixafor.

Our studies extend the findings of Shepherd *et al.* [14], who administered a single dose of AMD3100 (240 mcg/kg) to healthy subjects and reported significant increases in mononuclear cells, isolated from blood and expanded in culture conditions, that demonstrated angiogenic potential in NOD/SCID mice with hind-limb ischemia. We measured endothelial precursor cells by flow cytometry, using cell antigen markers currently used in clinical trial settings to select progenitor cells for autologous administration in patients with cardiovascular disease.

Several trials have examined the effect of the cytokine G-CSF, commonly used to mobilize CD34⁺ hematopoietic progenitor cells for transplant purposes, on the mobilization of progenitor cells in patients with coronary artery disease. We have reported that G-CSF, at a conventional mobilizing dose of 10 mcg/kg/day subcutaneously for 5 days, increased CD133⁺/VEGFR-2⁺ cells to approximately 1900 cells/mL [3]. The smaller number of CD133⁺/VEGFR-2⁺ cells mobilized in the current study may in part reflect different techniques in processing cells for flow cytometry between the two studies. For example, a greater effort was made to exclude dead cells during flow cytometry in the current study. The relatively small number of progenitor cells mobilized by plerixafor may be insufficient for homing to and repair of diseased cardiovascular tissue, but may be sufficient for collection by leukapheresis with CD133 selection. This strategy is currently under investigation in clinical trials, with G-CSF as the mobilizing agent.

Despite success in mobilizing progenitor cells with G-CSF, risks associated with G-CSF-induced inflammation of plaques in patients with atherosclerotic cardiovascular disease and unstable angina may preclude widespread use of this strategy. In a study of G-CSF administration to 16 patients with coronary artery disease and frequent angina, white blood cell counts increased approximately seven-fold from baseline (mostly granulocytes) and was associated with a significant increase in high sensitivity C-reactive protein levels, a marker of inflammation [4]. Two serious adverse events were reported in that study, including an acute non-ST elevation myocardial infarction 8 h after the fifth G-CSF injection in one subject, and a fatal myocardial infarction 17 days after treatment in another subject. Additional case reports of cardiovascular events, including death, have been reported for patients undergoing treatment with G-CSF [5–10].

Reports of significant increases in cell adhesion molecules, matrix metalloproteases, cytokines and cytokine receptors, serum amyloid A, hepatocyte growth factor and high sensitivity C-reactive protein levels in human subjects receiving G-CSF have raised concern regarding potential harm that may be associated with the marked leukocytosis and pro-inflammatory effects of G-CSF administration [4,15,16]. With the exception of a marginal increase in macrophage inflammatory protein-1 beta, we found the majority of inflammatory cytokines tested in our subjects did not increase following plerixafor administration. Importantly, systemic markers of inflammation high sensitivity C-reactive protein and serum amyloid A did not increase in association with the small increases observed with macrophage inflammatory protein-1 beta. This lack of activation of systemic inflammation could be an advantage for using this agent in patients with coronary artery disease, in whom plaque rupture promoted by inflammation may occur [17,18].

We conclude that plerixafor mobilizes BM-derived progenitor cells, under investigation for cardiovascular disease, into the circulation in healthy subjects without evidence of a systemic inflammatory response.

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Figure 1. CD133⁺ cells measured by flow cytometry, adjusted for absolute mononuclear cells in the blood sample, before and 4–6 h following plerixafor injection in healthy subjects.

Table I

White blood cell (WBC), lymphocyte and granulocyte levels before and 6 h after plerixafor injection in healthy subjects.

	Pre-	Post-	P-value
240 mcg/kg (<i>n</i> = 3)			
WBC (cells/mL)	7147±1719	21 633±3782	0.0303
Lymphocytes (cells/mL)	2473±696	5890±696	0.0129
Granulocytes (cells/mL)	5240±1855	12 850±2771	0.1238
320 mcg/kg (<i>n</i> = 5)			
WBC (cells/mL)	6620±748	19 760±1884	0.0005
Lymphocytes (cells/mL)	2028±341	5836±590	0.0008
Granulocytes (cells/mL)	3862±444	11 173±1354	0.0023
400 mcg/kg (<i>n</i> = 7)			
WBC (cells/mL)	5954±533	21 600±1357	<0.0001
Lymphocytes (cells/mL)	1914±154	6159±420	<0.0001
Granulocytes (cells/mL)	3379±522	12 370±1275	0.0002