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Effects of Granulocyte Colony Stimulating Factor on Monosomy 7 Aneuploidy in Healthy Hematopoietic Stem Cell and Granulocyte Donors

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Abstract

Background—Reports of monosomy 7 in patients receiving granulocyte colony stimulating factor (G-CSF) have raised concerns that this cytokine may promote genomic instability. However, there are no studies addressing whether repeated administration of G-CSF produces monosomy 7 aneuploidy in healthy donors.

Study Design and Methods—We examined chromosomes 7 and 8 by fluorescent in situ hybridization (FISH) in CD34+ cells from 35 healthy hematopoietic stem cell transplant (HSCT) donors after G-CSF administration for 5 days, and by spectral karyotyping analysis (SKY) in four individuals to assess chromosomal integrity. We also studied 38 granulocyte donors who received up to 42 doses of G-CSF and dexamethasone (Dex) using FISH for chromosomes 7 and 8.

Results—We found no abnormalities in chromosomes 7 and 8 in G-CSF mobilized CD34+ cells when assessed by FISH or SKY, nor did we detect aneuploidy in G-CSF/Dex treated donors.

Conclusion—G-CSF does not promote clinically detectable monosomy 7 or trisomy 8 aneuploidy in HSCT or granulocyte donors. These findings should be reassuring to healthy HSCT and granulocyte donors.

INTRODUCTION

Monosomy 7 is the second most common cytogenetic abnormality in myelodysplastic syndromes, and is associated with refractory cytopenias and progression to leukemia^{1,2}. Administration of granulocyte colony stimulating factor (G-CSF) to patients with severe aplastic anemia (SAA) and severe congenital neutropenia (SCN) is associated with an increased rate of monosomy 7 MDS and progression to AML in retrospective analyses^{3–5}, and prior studies have described transient changes in chromosomal integrity, aneuploidy, and tetraploidy after administration of G-CSF to healthy donors^{6–8}. Two reports have also

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implicated G-CSF as a potential risk factor for secondary MDS/AML after adjuvant chemotherapy for breast cancer^{9,10}. Collectively, these findings have raised concerns about the safety of G-CSF for mobilization of granulocytes and stem cells in healthy donors¹¹. On the other hand, others have found no increase in clonal evolution to MDS in patients with SAA and SCN treated with G-CSF compared to untreated patients^{12–14}.

We previously reported no new abnormalities in chromosomes 7 and 8 after *in vitro* culture of normal donor bone marrow mononuclear cells with pharmacologic doses of G-CSF¹⁵. However, there is a paucity of data on the effects of G-CSF mobilization on monosomy 7 aneuploidy, and there are no systematic studies addressing the long-term effects of G-CSF administration on chromosomal aneuploidy or in healthy donors treated with multiple doses of G-CSF. In the present study we evaluated aneuploidy in G-CSF mobilized allografts by FISH and SKY, and in healthy granulocyte donors exposed to multiple doses of G-CSF and dexamethasone. We demonstrate that G-CSF does not promote monosomy 7 chromosomal abnormalities in healthy HSCT donors or in granulocyte donors treated with G-CSF/Dex.

MATERIALS AND METHODS

Donor samples

Peripheral blood mononuclear cells (PBMCs) were isolated as previously described¹⁵ from 38 granulocyte donors and 36 healthy controls, and 35 CD34+ selected PBMCs were obtained from allogeneic stem cell transplant donors. Cells were cryopreserved in freezing media with 10% DMSO. Granulocyte donors last received doses of 5 mcg/kg G-CSF and 8 mg Dex at least six months prior to PBMC isolation (range 6 months to 9 years). CD34+ selected PBMCs were obtained from healthy allogeneic stem cell transplant donors mobilized with 10 mcg/kg G-CSF $\times$ 5 days. HSC samples were collected on the fifth day of G-CSF mobilization after cytapheresis and CD34+ selection. All samples were obtained from subjects on protocols approved by National Institutes of Health institutional review boards in accordance with the Declaration of Helsinki.

Cell culture

Cryopreserved mobilized CD34+ selected PBMCs were thawed and grown in Myelocult media (Stem Cell Technologies, Vancouver, BC) supplemented with myeloid growth factors and 400 ng/mL of G-CSF in six well plates at 37 degrees Celsius with 5% CO₂ as previously described¹⁵.

Interphase FISH

Cryopreserved PBMCs were thawed and nuclei were prepared and dropped onto slides using standard methods¹⁵. Nuclei were hybridized with orange and green centromeric chromosome 7 and 8 probes (Vysis, Downers Grove, IL), and imaged by fluorescence microscopy; 400 nuclei were counted on each slide.

Spectral karyotyping (SKY)

SKY was performed on four randomly selected healthy donor G-CSF mobilized, CD34+ selected peripheral blood stem cell (PBSC) samples. Metaphase chromosome suspensions were prepared by treating cells in hypotonic solution (0.075 mol/l KCl); next, the cells were fixed using methanol:acetic acid (3:1, vol/vol) and dropped onto slides in a humidity controlled chamber. The slides were aged at 37°C for approximately 1 week. Chromosome preparations were hybridized with SKY probes (prepared in-house) for 72 hours. The protocols for slide pre-treatment, denaturation, detection, and imaging have been published¹⁶. At least 20 metaphase spreads were analyzed per sample and scored for chromosome number (ploidy), as well as numerical and structural aberrations. Spectrum-

based classification and analysis of the fluorescence images was achieved using SkyView™ software (Applied Spectral Imaging). Karyotypes were characterized using the human chromosome nomenclature rules adopted in 2009 by the International System for Human Cytogenetics Nomenclature.

Statistics

Data obtained with cells from one donor were considered as one experiment (*n*). Statistical analyses included the calculation of mean, SE, and *p* values using GraphPad Prism software using Mann Whitney test to compare data sets with two-tailed *P* values. The significance level was set as *p* = 0.05, and the actual *p* values are indicated for each experiment.

RESULTS AND DISCUSSION

We previously reported that bone marrow mononuclear cells grown in a high concentration of G-CSF (400 ng/mL) exhibit expansion of occult monosomy 7 clones in patients with MDS, but G-CSF has no effect on bone marrow mononuclear cells from patients with severe aplastic anemia or healthy donors¹⁵. In our prior studies healthy donor controls exhibited aneuploidy levels of up to 4% due to technical artifacts such as incomplete probe hybridization.¹⁵ Previous studies have reported that G-CSF mobilization induces tetraploidy, aneuploidy, and alterations in replication timing in the peripheral blood of healthy HSCT donors⁶⁻⁸. However, these studies did not assess the effects of G-CSF on monosomy 7 aneuploidy. To examine the effects of G-CSF mobilization on chromosomes 7 and 8 (two MDS-associated chromosomal abnormalities) in healthy SCT donors, FISH studies were performed using centromere probes for chromosomes 7 (orange) and 8 (green) in 35 healthy G-CSF mobilized, CD34+ selected PBSC grafts (Fig. 1A). As shown in figure 1B, the percentage of monosomy 7 aneuploidy in CD34+ selected PBSCs was below the 4% detection threshold both before and after stimulation with G-CSF in culture for two weeks. Trisomy 8 aneuploidy was also undetectable in both unstimulated and stimulated CD34+ PBSCs (not shown). Previous studies by Nagler et al and Marmier-Savet et al reported that G-CSF mobilization is associated with aneuploidy of chromosomes 8 and 17 in healthy HSC donor PBSCs cultured with phytohemagglutinin to stimulate lymphocytes⁷⁻⁸. While neither study examined monosomy 7 aneuploidy, the lack of trisomy 8 in our study contrasts their findings. Our study differs from theirs in that we preferentially stimulated the myeloid and stem cell pool rather than lymphocytes prior to performing FISH. The presence of chromosomal aneuploidy in mitogen-stimulated effector lymphocytes is less concerning than aneuploidy within the CD34+ stem cell compartment. Indeed, Marmier-Savet et al demonstrated that loss of chromosome 17 is detectable in lymphocytes, but not in the CD34+ fraction of G-CSF mobilized donors⁸.

To further assess for chromosomal aneuploidy or rearrangements in G-CSF mobilized allografts, we performed spectral karyotype analysis (SKY) on PBSCs after G-CSF mobilization and CD34+ selection in samples from four randomly selected individual donors. A minimum of 20 metaphase spreads were analyzed for each donor sample. Chromosomal rearrangements were detected, including breakage on the short arm of chromosome 1 (depicted in figure 2), centromeric breakage of chromosome 9, breakage on the long arm of chromosome 4, and centromeric breakage of chromosome 2. However, none met the International System for Human Cytogenetics Nomenclature definition of clonal abnormalities, nor did we detect any clonal aneuploidy in CD34+ cells by SKY.

G-CSF is administered extensively to granulocyte donors who may receive serial doses of this cytokine over a lifetime. No previous report has examined the late effects of G-CSF on monosomy 7 aneuploidy in healthy donors. We quantitated monosomy 7 and trisomy 8 aneuploidy in 38 healthy granulocyte donors who had received serial doses of 5 mcg/kg G-

CSF (median= 12 doses; range 3–42) and dexamethasone (8 mg), and in 36 healthy controls. This cohort afforded us the opportunity to examine the steady state effects of G-CSF and Dex on monosomy 7 aneuploidy in healthy individuals. A single 300 mcg dose of G-CSF raises serum levels as high as 1000-fold above physiologic levels¹⁷. As shown in Figure 3, the percentage of monosomy 7 aneuploidy in G-CSF/Dex treated donors was the same as in untreated controls ($p= 0.5954$), and both were below the threshold of detection for FISH. Similarly, trisomy 8 aneuploidy was not detectable in control or G-CSF/Dex treated donors (not shown). To our knowledge, this is the first study examining the effects of G-CSF/Dex on monosomy 7 aneuploidy in healthy granulocyte donors.

Some limitations of our study design are that it does not allow us to assess the effects of G-CSF on chromosomal aneuploidy in the bone marrow, and our HSCT donor studies were performed immediately after a first or second round of G-CSF mobilization, so they may not be applicable to donors who undergo G-CSF mobilization on a more frequent basis. It is conceivable that small monosomy 7 clones may be induced by G-CSF in the marrow which we could not detect in circulating CD34+ HSCs. However, the chromosomal changes seen in previous studies were readily detectable in peripheral blood cells at the time of G-CSF mobilization^{6–8}. Furthermore, monosomy 7 MDS has a short latency from initial diagnosis to progression to acute leukemia², and emerging data from observational studies do not indicate an increased incidence of monosomy 7 AML in unrelated HSCT donors^{18;19}.

In summary, we observed neither monosomy 7 aneuploidy nor clonal loss of chromosome integrity in G-CSF mobilized, CD34+ selected PBMCs in unmanipulated cells, or after culturing the allografts in high concentrations of G-CSF in order to promote the expansion of occult aneuploid myeloid or stem cell clones.¹⁵ We also did not detect monosomy 7 or trisomy 8 aneuploidy in a cohort of granulocyte donors treated serially with as many as 42 doses of G-CSF/Dex. Our findings should be reassuring to healthy granulocyte and HSCT donors, and they are consistent with recent reports of no increase in myeloid malignancies in cohorts of unrelated stem cell^{18;19} and granulocyte donors²⁰ followed longitudinally.

Acknowledgments

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Fig. 1B

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Figure 1B. Effect of G-CSF mobilization on monosomy 7 aneuploidy in healthy stem cell donors. Percent of monosomy 7 aneuploidy was quantitated by FISH in CD34⁺ selected PBSCs from G-CSF mobilized healthy HSCT donors at baseline (unstimulated), and after stimulation with 400 ng/mL G-CSF (G-CSF stimulated). Bars represent means $\pm$ SEMs.

Figure 2. Spectral karyotype of PBSCs obtained from G-CSF mobilized healthy allograft donors
This image depicts a breakage on the short arm of chromosome 1 in one of twenty metaphases, but no clonal aneuploidy or structural chromosomal abnormalities was seen. Results are representative from 4 independent donor samples.

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