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REVIEW ARTICLE

Biologic and Clinical Effects of Granulocyte Colony-Stimulating Factor in Normal Individuals

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GRANULOCYTE colony-stimulating factor (G-CSF) is a hematopoietic cytokine produced by monocytes, fibroblasts, and endothelial cells.[1-3] G-CSF is known to have multiple functions in normal, steady-state hematopoiesis such as the regulation of neutrophil production and release from the bone marrow, neutrophil progenitor proliferation and differentiation, and the state of functional activation of neutrophils.[1-3] Genetically engineered recombinant human G-CSF, now available both in a nonglycosylated (filgrastim) and glycosylated (lenograstim) form, was introduced in phase I clinical trials about 8 years ago.[4] At pharmacologic doses ranging from 1 to 70 μg/kg/d, it was found to have reproducible biologic and clinical activity in various settings, such as chronic idiopathic neutropenia, chemotherapy-induced myelosuppression, recovery from aplasia after allogeneic or autologous marrow transplantation and mobilization of $CD34^+$ progenitor cells in the peripheral circulation with or without prior chemotherapy.[5] Its expanding use since then, occasionally for indications not clearly supported by available evidence, led recently to the formulation of recommendations on the use of hematopoietic colony-stimulating factors (G-CSF and granulocyte-macrophage colony-stimulating factor [GM-CSF]) by the American Society for Clinical Oncology.[6]

G-CSF at low doses (3.5 to 6 μg/kg/d) has been successfully administered with minimal toxicity to normal subjects to improve the yield of granulocyte collections by leukapheresis.[7-9] Similarly, G-CSF was well tolerated when administered to normal subjects to mobilize (and collect) peripheral blood progenitor cells (PBPCs) for syngeneic transplantation.[10] Based on such initial experience, over the past 2 years, the use of G-CSF in normal individuals has undergone a substantial increase, with the focus on mobilizing and collecting by leukapheresis PBPCs for allografting in HLA-identical and, to a lesser extent, HLA-nonidentical patients with hematologic malignancies.[11-15] Although preliminary reports seem to indicate an acceptable short-term toxicity profile despite doses up to 16 μg/kg/d,[11-15] the number of normal donors treated so far has been relatively small (particularly at the higher dose levels) and legitimate concerns remain about the short-term and particularly long-term safety of G-CSF. On the other hand, collecting granulocytes from G-CSF–mobilized normal donors is now a recognized blood banking procedure.[7-9] As allografting with G-CSF–mobilized PBPCs is gaining considerable momentum in transplant centers worldwide,[11-16] the time seems appropriate to review available information on the biologic and clinical effects of G-CSF administration in normal subjects. Because most of the currently available literature on G-CSF refers to filgrastim, in the remainder of this review the terms G-CSF and filgrastim will be used interchangeably, unless otherwise specified.

PHARMACOLOGY

The pharmacologic profile of G-CSF in normal donors does not differ significantly from the one documented in cancer patients.[5,17] G-CSF (filgrastim and lenograstim) can be administered subcutaneously or intravenously, although the subcutaneous route is usually preferred in normal, ambulatory individuals. Maximum serum concentrations after subcutaneous administration of filgrastim are reached within 2 to 8 hours.[17,18] Subcutaneous doses of 3.5 and 11.5 μg/kg result in maximum serum concentrations of 4 ng/mL and 49 ng/mL, respectively.[17] These serum levels (particularly the latter) are uncommonly reached by endogenous G-CSF in human subjects under physiologic or pathophysiologic conditions and only in extreme circumstances, such as cases of febrile neutropenia with gram-negative sepsis.[19-21] Twenty-four hours after subcutaneous administration, circulating G-CSF levels are still elevated above the baseline.[18]

The volume of distribution of filgrastim averages 150 mL/kg in normal subjects, with an elimination half-life of about 3 to 4 hours regardless of the route of administration.[17] Clearance rates are approximately 0.5 to 0.7 mL/min/kg, and no dose adjustment is currently recommended for abnormalities in renal or liver function or in the elderly.[17]

Clinically relevant drug interactions between G-CSF and other drugs have not been reported. Its effects on the devel-

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oping fetus have not been studied, and G-CSF administration during pregnancy is not recommended.[17] Data on G-CSF treatment in hematologically normal pediatric subjects acting as PBPC donors is very limited,[22] mostly because of the technical complexity of establishing an adequate vascular access and performing continuous-flow apheresis in normal blood progenitor cell donors with a small total blood volume (<2 L).

BIOLOGIC EFFECTS

Effects on neutrophil kinetics. The administration of G-CSF to normal subjects causes an increase in polymorphonuclear (PMN) leukocyte production and a dose-dependent reduction in their maturation time in the marrow, without a measurable impact on the blood PMN leukocyte survival or distribution between marginal and circulating pools.[23] In the marrow of normal individuals, G-CSF leads to an expansion of the myeloid compartment, mainly at the promyelocyte and myelocyte level.[24] Although the percentage of marrow myeloblasts seems unaffected by G-CSF administration in normal subjects,[24] some patients with myelodysplastic syndromes (MDS)[25] and acute myeloid leukemia (AML)[26] respond with an increase in marrow and/or peripheral blood blasts. Despite the marked neutrophilia, PMN leukocyte tissue migration seems reduced in G-CSF–treated normal subjects.[23]

Effects on neutrophil functional status. G-CSF is a powerful in vitro and in vivo activator for neutrophils obtained from normal donors.[18,27] G-CSF is capable of mobilizing secretory vesicles (leukocyte alkaline phosphatase and CD11b) and inducing the release of specific granules (lactoferrin, CD11b, and CD66b) and azurophil granules (elastase-α1antitripsin complexes).[18] In vitro exposure of normal marrow PMN leukocytes to G-CSF (lenograstim) has been reported to significantly increase the expression of the mRNA for alkaline phosphatase.[28]

PMN respiratory burst metabolism is enhanced in normal volunteers in response to various stimuli by G-CSF administration in a dose-dependent, age-independent fashion.[24] G-CSF has been shown in vitro to enhance superoxide release and membrane potential changes induced by receptor-mediated agonists such as N-formyl-methionyl-leucyl-phenylalanine and wheat germ agglutinin.[29,30] Exposure of normal human granulocytes to G-CSF also increases the expression of C3bi receptors and the adherence to nylon fibers.[30]

Effects on cytokine responses. The peripheral blood cytokine profile is substantially altered in normal subjects after G-CSF administration.[31,32] The most significant findings include an increase in interleukin-1 receptor antagonist (IL-1ra) release, an increase in the level of soluble tumor necrosis factor (TNF) receptors (p55 and p75), a reduction in TNF release induced by various stimuli, and an increase in IL-6, IL-8, and IL-10 release.[31] The release of GM-CSF and interferon-γ is reduced as well. These findings have been interpreted as a G-CSF–induced shift of the endogenous cytokine profile towards an antiinflammatory cytokine response.[31] In another study, G-CSF administration to normal volunteers increased the plasma levels of TNF-α, soluble TNF receptors (p55 and p75), and IL-1ra induced by the administration of endotoxin.[32] Interestingly, G-CSF has also been shown in vitro to downregulate allogeneic immune responses of peripheral blood mononuclear cells from normal individuals by inhibiting TNF-α production at a post-transcriptional level.[33]

Effects on circulating PBPCs. The ability of G-CSF, even at low doses, to consistently and substantially increase the number of circulating progenitor cells of multiple hematopoietic lineages is well known.[34-37] Less widely recognized is the fact that G-CSF in normal subjects mobilizes very primitive progenitors even to a greater extent than committed progenitors.[34,35,37] In a study on PBPCs obtained from G-CSF–treated normal individuals, the replating capacity of primary colonies from 5-week-old long-term culture (LTC) systems was found to be significantly higher than that from 5-week-old LTC initiated in a steady state.[34] In normal donors, a 5-day course of G-CSF increased the clonogenic potential of $CD34^+$ cells and shifted the phenotype of LTC-initiating cells from $CD34^+CD38^{-/dim}$ to $CD34^+CD38^{bright}$.[35] Low (2.5 μg/kg/d) daily doses of G-CSF (lenograstim) consistently induce a significant increase in the circulating colony-forming units granulocyte-macrophage (CFU-GM) and burst-forming units erythroid (BFU-E), which is paralleled by an increase in the level of $CD34^+$ cells.[36]

The administration of G-CSF causes a significant increase in the circulating $CD34^+$ cells and primitive subsets such as $CD34^+$ Thy-1^{dim} and $CD34^+$Thy-1^{dim} $CD38^-$. The percentage of $CD34^+$Thy-1^{dim} and $CD34^+$Thy-1^{dim} $CD38^-$ among $CD34^+$ cells increases as well, suggesting an additional peripheralization effect of G-CSF on primitive $CD34^+$ subsets.[37] When compared with the $CD34^+$ progenitor cell profile in the marrow, G-CSF mobilization causes a substantial increase in the percentage of circulating $CD34^+CD13^+$ and $CD34^+CD33^+$ cells (myeloid precursors) and a decrease in the percentage of circulating $CD34^+CD10^+$ and $CD34^+$ $CD19^+$ cells (B lymphocyte precursors).[38]

The kinetics of mobilization of $CD34^+$ progenitor cells in normal donors has been studied by several investigators. After daily administration of G-CSF, the peak level of circulating $CD34^+$ progenitor cells (ordinarily a 15- to 35-fold increase over baseline values) is usually reached around day 5,[38-42] with a subsequent decline thereafter despite continued G-CSF administration.[41,43] At least for G-CSF (filgrastim and lenograstim) doses up to 10 μg/kg /d, there is evidence for a dose-response effect with regard to the mobilization efficiency.[43-45] In one study, the peak of circulating CFU-GMs on day 5 of G-CSF was not reached until 6 to 30 hours after G-CSF (lenograstim) administration.[46]

Increasing age has been reported by some[24,42] but not others[41] to have a negative impact on the G-CSF–induced mobilization of PBPCs. These studies have included a relatively small number of donors who received different G-CSF doses. We recently analyzed a group of 119 donors mobilized at our institution with the same G-CSF dose (6 μg/kg subcutaneously twice daily) and including approximately 20% of subjects 50 years of age or older. A negative correlation was found between age and the apheresis yield of $CD34^+$ progenitor cells (Anderlini et al, unpublished data). However, the correlation is relatively weak, and we have per-

formed successful PBPC collections with one apheresis in the majority of normal donors up to 77 years of age (Körbling et al, unpublished data). The conflicting results in the literature are probably related to the small sample size and different doses of G-CSF used.

It is unclear whether the glycosylated (lenograstim) and nonglycosylated (filgrastim) forms of G-CSF differ with regard to their mobilizing activity. Recently reported data suggest that lenograstim may be biologically more active than filgrastim in mobilizing CFU-GMs and $CD34^+$ progenitor cells in normal individuals.[47]

Effects on hematologic and coagulation parameters. A 4- to 6-day course of G-CSF (filgrastim and lenograstim) in normal donors causes a dose-dependent neutrophilic leukocytosis with a marked left shift.[24,48,49] After single-dose subcutaneous administration, the neutrophil count peaks at approximately 12 hours after a transient initial neutropenic phase and remains above baseline values for about 2 to 3 days.[18,50] The level of neutrophilia is significantly enhanced by the concomitant administration of dexamethasone.[50] The degree of leukocytosis is dependent on the duration of G-CSF administration,[7,41,51] and no age-related difference in the PMN response to G-CSF has been shown.[24] On the average, after 3 to 4 days of filgrastim (6 μg/kg subcutaneously twice daily), measurement of blood counts show an eightfold increase in the number of neutrophils and monocytes and a twofold increase in the number of lymphocytes.[48] The hemoglobin level may decrease slightly.[7,48] We[48] and others[7,17] have also observed a slight but statistically significant decrease in platelet count at the peak of the G-CSF–induced leukocytosis. Although this reduction is small, it may be subsequently enhanced by an apheresis-related decrease in the platelet count.[48,52] A significant reduction in circulating platelets is a recognized complication of large-volume leukapheresis, in which the volume of blood processed equals three times (or more) the donor's blood volume.[48,52] A decrease of about 40% to 50% at the end of each procedure that may require 1 week (or longer) to normalize has been reported.[48,52] Thus, borderline-low baseline platelet counts and/or consecutive procedures may result in clinically relevant (albeit self-limiting) thrombocytopenia ($<100 \times 10^9$/L).[48]

The impact of G-CSF administration on the coagulation system of normal PBPC donors has not been adequately studied. A recent report suggests that even a brief course (5 to 7 days) of G-CSF (15 μg/kg/d) has a significant (albeit transient) impact on several hemostatic system variables, namely plasma hypercoagulation markers, plasma markers of endothelial damage, and monocyte procoagulant activity, and may therefore potentially induce a prothrombotic state.[53] However, some of these findings have been questioned.[54] No thrombotic events or related clinical manifestations of hypercoagulability have been reported to date during G-CSF administration in normal subjects.

Effects on chemistry parameters. The G-CSF–induced leukocytosis is accompanied by a marked, reversible increase (about twofold to threefold) in the serum levels of alkaline phosphatase (AP) and lactate dehydrogenase (LDH).[17,42,48] The serum levels of gamma glutamyl transferase and serum creatine phosphokinase remain within the normal range, suggesting that the AP and LDH elevation is likely related to the expanding myeloid cell mass.[48] Measurement of the serum LDH isoenzyme profile shows that the LDH elevation involves predominantly the LDH-4 and LDH-5 fractions.[48]

A mild but significant decrease in the serum potassium and magnesium level was also found in some donors at the peak of the G-CSF–induced leukocytosis.[42,48] Electrolyte supplementation has been administered in some instances,[48] mainly to prevent or minimize the possibility of electrolyte imbalances that can be encountered after large-volume leukapheresis (M. Körbling, personal observation). An increase in the serum uric acid level[48] and a transient decrease in serum cholesterol[54] have been noted as well.

SHORT-TERM CLINICAL TOXICITY

Several studies of G-CSF (filgrastim and lenograstim) treatment of normal apheresis donors have now been presented or published, although not all of them have included detailed clinical toxicity data. A summary of the doses used and adverse events published for approximately 400 normal subjects treated at various institutions worldwide is shown in Table 1.

Although the duration of G-CSF administration has been fairly constant (4 to 6 days), the doses used have been much more variable (2 to 16 μg/kg/d), usually administered subcutaneously once daily. Lower doses (eg, up to 5 to 6 μg/kg/d or every other day) are considered adequate to collect granulocytes,[7-9,50] whereas higher doses ($\geq$10 μg/kg/d) have been ordinarily used to collect PBPCs.[11-15] However, the optimal dose and schedule of administration of G-CSF in both settings have not been established. In general, the adverse events reported are similar to the ones reported by cancer patients.[5] The adverse events consist mainly of bone pain, headache, fatigue, and nausea. Anxiety, noncardiac chest pain, myalgias, insomnia, night sweats, skin rashes, anorexia, dizziness, weight gain, local reactions at the injection site, and vomiting have occasionally been reported as well, whereas G-CSF–related fever has been rare. These adverse events have been described as moderate-to-severe (grade 2 to 3 NCI common toxicity criteria) by about 60% of the donors receiving 12 μg/kg/d.[58] Discontinuation of G-CSF administration for intolerable side effects has not, in our experience, been necessary, although it occurred in 3% of the donors in one series.[56] These adverse events usually resolve within a few days of G-CSF discontinuation, although in some cases they have persisted for 1 week or longer.[48]

G-CSF–related side effects can usually be managed successfully with minor analgesics (eg, acetaminophen and ibuprofen) and have only infrequently required opiates (eg, codeine) for bone pain and/or headache relief.[58,59] In one recent study, the concomitant administration of dexamethasone did not ameliorate G-CSF–related adverse events.[50] The antihistamine astemizole has recently been reported to be effective in a patient with G-CSF–induced bone pain unresponsive to acetaminophen.[60] At our institution, none of these donors has required hospitalization. Several groups have reported

Table 1. Summary of Clinical Toxicity of G-CSF in Normal Apheresis Donors

Reference	No. of Donors	G-CSF Dose and Schedule*	Bone Pain (%)	Headache (%)	Fatigue (%)	Miscellaneous (%)
Sato et al[46]†	15	2 μg/kg/d for 1-5 d	60 (low back pain)			Rash/fever (7)
Matsunaga et al[36]†	3	2.5 μg/kg for 6 d, then 5 μg/kg for 4 d	66	66		
Suzue et al[49]†	9	2-5 μg/kg/d for 5 d	100	100	100	Chest pain (80), vertigo and myalgia (40), anorexia (20) at the higher dose
Bensinger et al[7]	8	3.5-6 μg/kg/d for 9-14 d	25			
Bishop et al[55]	25	5 μg/kg/d for 6 d	76	52		Fever (28), chills (20)
Russell et al[14]	14	4.4-7.5 μg/kg/d for 2-4 d	100			Flu-like symptoms (43)
Stroncek et al[56]	62	2-10 μg/kg/d for 5 d	87; 90% taking analgesics at the higher dose level (n = 20)	35	18	Nausea (15), local reaction (10), night sweats (6), insomnia (6), dyspnea (3).
Grigg et al[41]	28	3-10 μg/kg/d for up to 10 d	93; 86 at the higher dose level (n = 15)	33	63	Dizziness (20), flu-like symptoms (17), muscle pain (17), hyperventilation (3).
Dreger et al[42]	9	5-10 μg/kg/d for 5 d	100 at 10 μg/kg/d (n = 6)			
Schmitz et al[12]	8	5-10 μg/kg/d for 5-6 d	66 at 10 μg/kg/d (n = 6)			
Azevedo et al[15]	17	10 μg/kg/d for 5 d	100 (all donors taking minor analgesics)	100		
Lane et al[57]	8	10 μg/kg/d for 4 d			100	Myalgia (100)
Link et al[39]	10	5 μg/kg/d bid for 5 d	70	70		
Kadar et al[54]	29	5 μg/kg bid for 5-7 d	90	11	6	Sleep disturbances (3)
Körbling et al[37]	41	6 μg/kg bid for 4–6 d	63	76		Ankle swelling (13), fluid retention/weight gain (28)
Anderlini et al[58]	77	6 μg/kg bid for 4-6 d	82 (69% of donors taking minor analgesics)	69	35	Nausea (10)
Bensinger et al[13]	8	16 μg/kg/d for 5 d	25			
Weaver et al[10]	4	16 μg/kg/d for 5 d	100			
Weinthal et al[59]	19	16 μg/kg/d for 5 d	47 (ostealgia requiring narcotic analgesia or headache)			Nausea (10)

Abbreviation: bid, twice daily.

* Administered by subcutaneous injection.

† Glycosylated G-CSF (lenograstim).

on a relationship between increasing G-CSF (filgrastim and lenograstim) dose and frequency and severity of side effects.[40,49,56] The duration of G-CSF administration has also been shown to influence, albeit to a relatively minor degree, the short-term toxicity profile.[51]

LONG-TERM EFFECTS

The long-term effects of even a brief course of G-CSF in normal individuals are presently unknown and can be clarified only with a longer follow-up. However, to address this issue formally, follow-up data on a large cohort of normal donors will be required, and an appropriate control cohort (ie, normal marrow donors) should ideally be used. It has been estimated that, to detect a 10-fold increase in leukemia risk for healthy PBPC donors (a substantial risk increase), more than 2,000 donors would need to be observed for 10 years or longer.[61] The logistics of this endeavour are obviously challenging and can be approached only by multicenter groups or registries. Such a system for donor follow-up is currently being discussed by the European Group for Blood and Marrow Transplantation (A. Gratwohl, personal communication; 1st International Symposium on Allogeneic Peripheral Blood Progenitor Cell Transplantation, Geneva, Switzerland, October 1995) and the International Bone Marrow Transplant Registry (M. Horowitz, personal communication, May 1996).

Data on the long-term safety of chronic (4 to 6 years) filgrastim treatment in severe congenital neutropenia (SCN) have recently been published.[62] Several cases of AML and MDS diagnosed during long-term G-CSF (filgrastim and lenograstim) treatment of patients with SCN and aplastic anemia (AA) have been reported.[62-66] It should be pointed out that SCN and AA patients may be predisposed to the development of AML and MDS as part of the natural history of their disease, and, at least for AA, this has been convincingly proved.[67] Therefore, the relevance of these observations with regard to normal subjects treated for only 4 to 6 days is unclear. In the majority of SCN patients, G-CSF was well tolerated. One interesting and probably unexpected finding

was the high incidence of osteopenia/osteoporosis.[62,68] In keeping with this, it has recently been reported that G-CSF treatment of normal donors results in the mobilization of osteoclast progenitors in the peripheral blood.[69]

A theoretical concern is the fact that, in normal individuals who are genetically HLA-identical with patients with an hematologic malignancy (eg, acute leukemia), the administration of a myeloid growth factor (although only for a brief period) may potentially uncover any underlying genetic predisposition to develop a similar disease. Moreover, in view of his/her sibling or parent status, the donor may also have been exposed to the same environmental factors possibly related to the development of leukemia in the patient. The results of a large survey of HLA typing (published in 1987) have shown nonrandom genetic differences in the incidence of leukemia, although the increase in relative risk was small (approximately twofold) and confined to the Cw3 and Cw4 antigens.[70] To our knowledge, a similar study using more contemporary HLA typing techniques has not been reported, but would certainly prove very valuable to address this issue.

CONCLUSION

On the basis of currently available data, G-CSF appears to have reproducible biologic activity and an acceptable short-term safety profile in normal subjects. However, experience remains limited, particularly at high doses ($\geq$10 μg/kg/d). Available data on the safety and efficacy of G-CSF administration in normal children and elderly subjects is also scarce. It should be emphasized that, although G-CSF is a physiologic substance, the serum levels and biologic effects achieved with these doses should be considered pharmacologic. Its use should be limited to brief (3 to 6 days) courses administered in institutions or by physicians familiar with the drug, and after an appropriate informed consent has been obtained. The consent form should summarize the data currently available on G-CSF safety in normal donors.

NOTE ADDED IN PROOF

An effect of G-CSF administration on the surface expression of effector cell molecules on normal monocytes, possibly medicated by secondary cytokine release, has been described.[71] In a recent study, ADP- and collagen-induced platelet aggregation were found to be increased in normal subjects after G-CSF administration.[72] This is in keeping with data previously reported by the same investigators, suggesting the presence of functional G-CSF receptors on normal platelets.[73,74] A case of acute iritis[75] and one of episcleritis[76] occurring in normal apheresis donors during G-CSF administration have been reported.

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