A Comparison of Therapeutic Schedules for Administering Granulocyte Colony-Stimulating Factor to Nonhuman Primates After High-Dose Chemotherapy


Granulocyte colony-stimulating factor (G-CSF) has been shown to be effective in clinical trials for reducing the period of neutropenia after chemotherapy. In this study, we compared the timing for initiating G-CSF administration after chemotherapy with the duration of neutropenia and hematopoietic regeneration. Nonhuman primates treated with high-dose chemotherapy (mechloroethamine, 1.5 mg/kg, intravenously) and not administered G-CSF therapy experienced 8 days of neutropenia (absolute neutrophil count [ANC] <1,000/mm³) and had an ANC nadir of 124 ± 64/mm³ at day 7. Monkeys receiving G-CSF (5 μg/kg/d, subcutaneously) began treatment on either days 1, 3, 5, or 7 after chemotherapy. Monkeys treated with G-CSF had an earlier ANC recovery and the number of days with an ANC <500/mm³ and ANC <1,000/mm³ was reduced by approximately 50% in all treatment strategies. All G-CSF-treated animals, irrespective of the time that G-CSF was initiated, reached an ANC of 10,000/mm³ on day 13 ± 1 day after chemotherapy. These results demonstrated that the duration of G-CSF therapy was almost twice as long for monkeys treated on day 1 as it was for monkeys that received therapy beginning on day 7. A comparison of the results for all treated monkeys identified a distinct difference in the responses of monkeys treated on day 1 from that of animals treated with G-CSF at later times. G-CSF initiated 1 day after chemotherapy led to an earlier onset of neutropenia and a more rapid and augmented recovery of myeloid progenitor cells in the peripheral blood when compared with control and delayed therapy groups. This study demonstrates that neutropenia due to a single dose of mechloroethamine can be equally reduced with both early and delayed initiation of G-CSF. Further, initiating G-CSF therapy after 7 days required approximately 50% less days of therapy to reach an appropriate termination point. The applicability of these findings to other chemotherapy regimens and for repeated cycles is uncertain and needs to be further evaluated.

This is a US government work. There are no restrictions on its use.

G RANULOCYTE colony-stimulating factor (G-CSF) is one of a family of glycoprotein hormones that play a role in mediating the proliferation, differentiation, and functional capacity of blood and bone marrow cells. Specifically, G-CSF has been demonstrated to induce the proliferation of myeloid cells, enhance the functions of mature neutrophils, and accelerate the recovery of neutrophil counts following a variety of radiotherapeutic or chemotherapeutic regimens and after bone marrow transplantation.

The capability of G-CSF to stimulate the proliferation of hematopoietic progenitors and increasing the production of neutrophils in normal and chemotherapy-treated subjects holds promise for reducing the risk of infection after chemotherapy and potentially allowing the escalation of chemotherapy doses. However, the optimal timing for growth factor use in relationship to radiotherapy or chemotherapy is unknown. G-CSF therapy after melphalan has been shown to be efficacious in abrogating neutropenia when given as early as 24 hours or even as late as 8 days after chemotherapy. However, in animal studies the earlier application of G-CSF after lethal doses of radiation has proven to promote early neutrophil recovery and survival. A comparison of the clinical aspects of G-CSF therapy with respect to time of initiation has not been reported.

Growth factor therapy in reported clinical trials has been terminated after a preselected number of days of treatment or on reaching an established absolute neutrophil count (ANC). In these studies, the termination of growth factor has been associated with a dramatic decrease in the ANC, the clinical significance of which is unknown. Although no adverse effects from this steep decline of the ANC have been reported, we sought to determine if a tapering dose of G-CSF would ameliorate the severity of the decrease in neutrophils after therapy termination.

In the current study, we used high-dose mechloroethamine, an alkylating agent with suppressing effects on the hematopoietic and immune systems, to induce bone marrow hypoplasia and neutropenia in nonhuman primates. Four different schedules for initiating G-CSF therapy and two different discontinuation strategies were evaluated to compare the effects on the ANC and hematopoietic progenitor cells for the different regimens.

MATERIALS AND METHODS

Animals. Domestic born male rhesus monkeys, Macaca mulatta, ranging in weight from 6 to 11 kg were housed in individual stainless steel cages in conventional animal facility holding rooms. Monkeys were provided with commercial primate chow, fruit, and water ad libitum. Research was conducted adhering to the principles outlined in the Guide for the Care and Use of Laboratory Animals, prepared by the Institute of Laboratory and Animal Research, National Research Council.

From the Hematology/Oncology Division, US Naval Hospital, San Diego; AMGEN Inc, Thousand Oaks, CA; Immune Cell Biology Program, Naval Medical Research Institute; and Naval Medical Research and Development Command, Bethesda, MD.

Submitted August 9, 1991; accepted December 30, 1991.

Supported by the Naval Medical Research and Development Command, Research Task No. MM0095.003.1007.

Views presented in this report are those of the authors: no endorsement by the Department of the Navy has been given or should be inferred.

Address reprint requests to R.L. Monroy, PhD, Immune Cell Biology Program, Naval Medical Research Institute, Bethesda, MD 20889-5055.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

This is a US government work. There are no restrictions on its use. 0086-4971/92/7909-0004$0.00/0
Clinical care. Blood drawing and intravenous (IV) line insertion as required in accepted protocols were performed with the monkey sedated (ketamine HCl, 10 mg/kg, intramuscular [i.m.]). Bone marrow (BM) aspirations were performed on monkeys that were sedated and then anesthetized with thiamylal sodium (4% Biotal; Biocautics, St Joseph, MO). BM samples were aspirated aseptically from the posterior horn of the iliac crest and alternate sides were sampled during the evaluation period. Monkeys were clinically monitored daily. Blood and BM sampling was performed at periodic timepoints. Prophylactic cefotaxime (20 mg/kg twice per day, i.m.) was administered when the ANC was <500/mm³ and continued until the ANC was >500/mm³. Platelet and red blood cell transfusions were not required in this study.

Chemotherapy. Sedated primates were further premedicated with acepromazine (5 mg/kg, i.m.; PromAce, Aveco, Fort Dodge, IA) before chemotherapy was infused. Methotrexate HCl (Merck, Sharp & Dohme, West Point, PA) was reconstituted with sterile saline and administered immediately at a dose of 1.5 mg/kg through a continuously flowing IV saline line. The drug was delivered over 5 minutes.

G-CSF. Recombinant human G-CSF produced by Escherichia coli was supplied by AMGEN (Thousand Oaks, CA). Recombinant human G-CSF is a single-chain nonglycosylated polypeptide of molecular weight ~18.8 Kd.² It is formulated in open-label vials as a sterile solution at a concentration of 0.53 mg/mL. Each injection was prepared fresh daily and was administered to monkeys once per day, subcutaneously, at a dose of 5 μg/kg of body weight. G-CSF therapy was initiated either on 1 day (n = 3), 3 days (n = 1), 5 days (n = 2), or 7 days (n = 3) after chemotherapy. Three additional animals received chemotherapy but no G-CSF. G-CSF was continued until the ANC reached 10,000/mm³. On reaching this level, therapy was either discontinued abruptly (no further doses administered) or by a two-step taper with a 50% reduction (2.5 μg/kg/d) in the initial dose for 3 days followed by an additional reduction to 25% (1.25 μg/kg/d) of the original dose for 3 more days, after which therapy was discontinued.

Hematologic profile. To establish baseline hematologic values, peripheral blood (PB) was sampled at least three times over a period of several weeks before initiating chemotherapy. At each sampling time, PB (3 mL) was obtained from the saphenous vein and a complete hematologic profile with manual differential and reticulocyte counts was performed. Blood samples were taken in the morning before the administration of G-CSF.

Hematopoietic progenitor cell assays. PB and BM progenitor cell assays were performed on days 7, 14, 21, and 28 after chemotherapy. Assays were also performed on baseline samples. PB or BM samples were mixed with heparin (100 U/mL) and low-density cells were isolated by isopycnic centrifugation over Ficoll-paque (Pharmacia LKB Biotechnology Inc, Piscataway, NJ) (40 g for 30 minutes). Isolated low-density cells were plated in triplicate at either 1.25 × 10³/mL for PB or 2.5 × 10³/mL for BM in 35-mm culture dishes. Culture media used was Iscove’s Modified Dulbecco’s Medium (GIBCO, Grand Island, NY) supplemented with 30% heat-inactivated fetal calf serum, 5% conditioned media from 5637 cells (5X concentrate), recombinant erythropoietin, 1 U/mL (TCEpo; AMGEN, Thousand Oaks, CA), granulocyte-macrophage CSF (GM-CSF) CSF (40 U/mL, Genetics Institute, Cambridge, MA), and 0.9% methylcellulose. Cultures were incubated at 37°C, 5% CO₂ for 14 days. Colonies with 50 cells or greater were counted. Individual colonies of morphologic interest were harvested from methylcellulose cultures, cytospin cell preparations were prepared and stained with Hema-3 (Curtin Matheson Scientific Inc, Houston, TX). Diff-Quik. The frequency of colony-forming unit-granulocyte-macrophage (CFU-GM) per milliliter of PB was calculated using CFU-GM/1.25 × 10⁶ multiplied by the absolute number of mononuclear cells per milliliter of PB.

Statistical evaluation. The significance of the difference between mean values was determined by Student’s t-test.

RESULTS

Effect of G-CSF on ANC. Neutropenia was induced in all monkeys by high-dose mechloethamine. In control monkeys, the duration of neutropenia (ANC < 1,000/mm³) was 8 days with an ANC nadir of 124 ± 64/mm³ on day 7 (Fig 1). G-CSF therapy started 1 day after chemotherapy resulted in a more rapid decrease in ANC than observed for controls, reaching an earlier and lower nadir, 60 ± 30/mm³, on day 5 (Fig 1A). Despite the swifter decrease in the ANC of treated monkeys, neutrophils reappeared earlier than observed in untreated monkeys. The ANC of monkeys treated on day 1 began to increase after day 5, while untreated monkeys continued to decrease until the nadir at day 7.

Delaying G-CSF therapy to 3, 5, or 7 days after chemotherapy did not cause an early nadir as observed in day 1 treated monkeys (Fig 1, B and C). The observed decrease in ANC was similar to controls. The duration of neutropenia in these later G-CSF–treated monkeys was also reduced when compared with controls. A comparison of the number of days the animals were neutropenic for the various G-CSF therapy strategies evaluated is summarized in Table 1. For all G-CSF–treated monkeys, the number of days with the ANC < 500/mm³ and < 1,000/mm³ was reduced by approximately 50%.

Analysis of the time monkeys reached an ANC ≥ 10,000/mm³ led to the finding that most G-CSF–treated monkeys reached this ANC between 12 and 14 days (mean day = 13, standard deviation = 1, n = 9) irrespective of the time G-CSF therapy was initiated. Furthermore, a comparison of the number of days of G-CSF therapy each animal received before the ANC was > 10,000/mm³ (Table 2) demonstrates that the animals treated at the earlier times required approximately twice the amount of G-CSF to reach this predetermined, therapy termination neutrophil count.

Cessation of therapy. Paired and similarly treated animals were evaluated to determine whether a brief stepped reduction in the quantity of G-CSF administered would slow the decrease in neutrophil counts. The ANCs of a representative pair of G-CSF–treated monkeys as well as untreated controls are depicted in Fig 2. The ANC of animals in which therapy was abruptly terminated increased for 24 to 48 hours after G-CSF was discontinued. Following this, the ANC decreased rapidly, falling ~50% within 2 days and reaching an elevated ANC plateau. In no case did the ANC decrease to subnormal levels and no infectious complications were noted. In contrast, a two-step decreasing taper of G-CSF over 6 days delayed but did not eliminate the rapid decrease in neutrophil counts. The ANC continued to increase during the first 3 days at 50% of the dose. This was followed by a high ANC plateau maintained during the subsequent 3 days of G-CSF administration at 25% of the original dose. When G-CSF therapy
was terminated a rapid decline ensued, ANC decreasing 50% within 2 days. Again no adverse clinical consequences were noted.

**BM and PB progenitor cell activity.** Chemotherapy reduced CFU-GM activity in the PB and BM to undetectable levels after 24 hours (data not shown). The concentration of CFU-GM per milliliter of PB from untreated animals on day 7 was 11 ± 8, while monkeys treated with G-CSF beginning on day 1 had a significantly (*P* < .05, Student’s *t*-test) higher CFU-GM levels of 221 ± 165. The PB CFU-GM recovered to supranormal levels by day 14 in control and treated subjects but the recovery was further augmented in subjects receiving G-CSF. In comparison with control animals that reached a mean CFU-GM per milliliter of PB of 3,016 ± 897, monkeys receiving G-CSF on days 1, 5, and 7 reached mean CFU-GM per milliliter of PB of 11,994 ± 5,122, 4,781 ± 2,318, and 6,233 ± 757, respectively (Fig 3). The CFU-GM progenitor cell levels for day 1 and 7 treated animals were significantly greater (*P* < .01, Student’s *t*-test) than controls. All animals treated and controls had similar progenitor cell concentrations after 21 days. In contrast to the results from the PB progenitor cells, the CFU-GM levels in the bone marrow were not significantly different between the G-CSF–treated and control monkeys (Table 3).

**Effect of G-CSF therapy on other PB cells.** Lymphocyte counts reached a nadir of 360/mm³ 3 days after chemotherapy and remained at the nadir level until day 10. There were no differences in the kinetics of lymphocyte decrease and recovery between G-CSF–treated monkeys and controls (data not shown). Reticulocyte counts decreased to nearly undetectable levels 24 hours after chemotherapy and remained suppressed for 12 days. Recovery of reticulocyte counts occurred similarly in treated and control animals.

The administration of G-CSF had a subtle effect on platelet counts after chemotherapy. PB platelet counts of control animals reached a nadir of 61,000 ± 9,000/mm³ at day 10 after chemotherapy, whereas day 10 nadirs for the day 1, 3, 5, and 7 G-CSF–treated groups were 116,000 ± 43; 115,000; 155,000 ± 113,000; and 114,000 ± 77,000/mm³, respectively. The platelet counts of monkeys receiving

---

**Table 1. Effect of G-CSF and Timing of Initiating Therapy on the Duration of Neutropenia After Chemotherapy**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Days of ANC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 3)</td>
<td>No. of Days of ANC</td>
</tr>
<tr>
<td></td>
<td>&lt;500/mm³</td>
</tr>
<tr>
<td>G-CSF therapy</td>
<td></td>
</tr>
<tr>
<td>Day 1 (n = 3)</td>
<td>2.5</td>
</tr>
<tr>
<td>Day 3 (n = 1)</td>
<td>3.0</td>
</tr>
<tr>
<td>Day 5 (n = 2)</td>
<td>3.0</td>
</tr>
<tr>
<td>Day 7 (n = 3)</td>
<td>2.5</td>
</tr>
</tbody>
</table>

---

**Fig 1.** A comparison of the different G-CSF therapy schedules on the recovery of neutrophils in the PB after high-dose chemotherapy. The mean ANC of monkeys treated with G-CSF beginning on day 1 (○) (A); day 3 (△) (B); day 5 (●) (B); and day 7 (●) (C). The mean ANC of untreated control monkeys is depicted in all graphs (○). Each datapoint is the mean value with a standard deviation.
Table 2. Comparison of the Number of Days of G-CSF Therapy Required Before the ANC > 10,000/mm³ With the Day of Initiating Therapy

<table>
<thead>
<tr>
<th>Day of Therapy Initiated</th>
<th>No. of Days of Therapy Until ANC &gt; 10,000/mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13 ± 1.4</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>7</td>
<td>6 ± 0</td>
</tr>
</tbody>
</table>

Values are the means ± standard deviation of the mean.

G-CSF on day 1 were the only counts from the treated monkey groups that were significantly higher when compared with controls (P < .025, Student's t-test). However, a larger number of animals would have to be evaluated before any definite conclusion could be made with respect to the effect of G-CSF therapy on platelet production.

DISCUSSION

We demonstrated in a comparative study that the initiation of G-CSF therapy could be delayed up to 7 days after high-dose chemotherapy and still provide an equivalent reduction in the duration of neutropenia that is achieved when G-CSF is started 1 day after chemotherapy. Morstyn et al. reported similar findings following high-dose melphalan, where initiation of G-CSF 8 days after chemotherapy still resulted in an increase in ANC sufficient to abrogate neutropenia. Other studies comparing the time of initiating G-CSF after chemotherapy with the clinical endpoint, amelioration of neutropenia, have not been reported.

After high-dose mechlorethamine, G-CSF therapy did not eliminate neutropenia in any of the schedules evaluated and the depression of the neutrophil nadir did not differ significantly among the control group or any of the treatment groups. However, the kinetics of the neutrophil decrease did differ among the various groups. Animals treated with growth factor on day 1 had an earlier ANC nadir when compared with either controls or animals receiving G-CSF at later times. A similar observation was reported in other studies. In phase I/II clinical trials, the administration of G-CSF 1 day after melphalan therapy led to an earlier nadir than observed for control patients. In murine studies, the administration of G-CSF 24 hours after chemotherapy led to the onset of neutropenia 2 days earlier than observed in control animals. This phenomena of early neutrophil nadirs associated with initiation of G-CSF therapy early after chemotherapy has been attributed to G-CSF inducing the release of marrow neutrophils and accelerating the maturation of committed immature myeloid cells. Thus, the available neutrophil and late myeloid progenitor pool is rapidly exhausted and more immature BM myeloid progenitor cells ablated by chemotherapy are unable to provide replacement neutrophils. Initiation of G-CSF at later times did not exhaust the neutrophil pool beyond what has already been lost due to attrition. Indeed, the data show two apparent groupings with respect to the

Table 3. Effect of Different Initiation Strategies on CFU-GM Progenitor Cell Activity in the BM After Chemotherapy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>58 (18)</td>
<td>8 (4)</td>
<td>155 (51)</td>
<td>76 (3)</td>
</tr>
<tr>
<td>G-CSF day 1:</td>
<td>52 (14)</td>
<td>17 (6)</td>
<td>130 (42)</td>
<td>82 (2)</td>
</tr>
<tr>
<td>G-CSF day 5:</td>
<td>75 (17)</td>
<td>1 (1)</td>
<td>172 (35)</td>
<td>54 (23)</td>
</tr>
<tr>
<td>G-CSF day 7:</td>
<td>56 (20)</td>
<td>14 (7)</td>
<td>155 (9)</td>
<td>82 (31)</td>
</tr>
</tbody>
</table>

Results are presented as the mean number (± SEM) of CFU-GM colonies per 25,000 low-density BM cells plated.
COMPARISON OF G-CSF THERAPEUTIC SCHEDULES

kinetics of neutrophil decrease: day 1 treated monkeys and all the others treated at later times.

Interestingly, a rapid ANC recovery was observed shortly after initiating G-CSF on day 7. The ANC of monkeys treated with G-CSF on days 3 and 5 did not begin to increase until a similar time after chemotherapy. These results suggest that the functional integrity of progenitor/precursor cells capable of responding to G-CSF was not fully present until 8 to 10 days after chemotherapy. In other words, the presence of G-CSF-responsive progenitor cells is required before neutrophil expansion can begin to occur. This rapid increase in ANC was first described for nonhuman primates administered G-CSF after cyclophosphamide. In contrast to the response of monkeys in which G-CSF therapy was initiated on days 3 or 5, our results indicate that a 7-day delay in initiating therapy did not result in early neutrophil recovery and no animals survived. Thus, it appears that early initiation of G-CSF may have beneficial effects on BM progenitor cells that are not seen with later initiation of therapy.

One possible explanation of these results and those of the other studies cited is that G-CSF has beneficial effects (either directly or indirectly) on the BM in which progenitor/stem cell populations are either spared or helped to recover more efficiently from the effects of chemotherapy. A similar theory has been advanced by Schuening et al to account for G-CSF's beneficial effects after lethal radiation. The results of their study suggest that the survival and associated early neutrophil recovery was due to a positive effect of G-CSF at the level of the pluripotent stem cell. Whether the effect was direct or indirect through an accessory cell is uncertain. Additional studies administering G-CSF to mice exposed to an LD90 dose of radiation demonstrated that survival was directly correlated with the time G-CSF treatment was initiated. The earlier the administration of G-CSF the more significant was the improvement in survival. The enhanced survival was also correlated with improved recovery of endogenous spleen colonies, a hematopoietic indicator of recovery. In the present study, monkeys treated with G-CSF on day 1 showed an increase in the concentration of PB myeloid progenitor cells at days 7 and 14 compared with controls or to delayed G-CSF-treated groups. However, the frequency of progenitor cells in the BM was not significantly different between the various groups. Thus, G-CSF therapy within 24 hours after exposure to either radiation or chemotherapy has a positive effect on the recovery of progenitor cells. A delay in G-CSF administration did not have this benefit. Our data showing earlier recovery of neutrophils and augmented recovery of PB CFU-GM progenitor cells support the hypothesis of a beneficial effect from early G-CSF on early BM precursor cells that was not seen with later G-CSF therapy. The clinical significance of this finding for patients undergoing single or repeated cycles of chemotherapy is unknown because an equivalent reduction in neutropenia can be achieved with either early or delayed G-CSF schedule.

The results of our comparative studies have also delineated that the number of days G-CSF therapy was required was dependent on the day treatment was initiated. The total duration of therapy required was reduced to 6 days in monkeys not treated until day 7 versus 13 days of therapy in monkeys treated beginning 1 day after chemotherapy. This difference in duration of therapy was not a function of the preselected termination point, ANC > 10,000/mm3. For example, if a different termination point had been selected (eg, ANC > 5,000/mm3), then the duration of therapy would have been 11 days for the day 1 strategy and only 5 days for the day 7 strategy. Thus, delaying the administration of G-CSF until day 7 after chemotherapy decreased the number of days of therapy required by approximately 50% while still achieving the same reduction in the duration of neutropenia. These results are based on a clinically relevant single dose of G-CSF, but it is possible that by increasing the dose of G-CSF, a difference in the time to ANC > 10,000/mm3 could be detected between the different initiation schedules. Subtle differences between the various schedules could exist as a result of blood cell counts not being performed on a daily basis. However, the biologic response to G-CSF was reproducible within each small group of animals and the data consistently reflect the need for less G-CSF therapy in the later-treated animals.

This study has shown that G-CSF can be successfully used to ameliorate neutropenia that follows single-cycle, high-dose mechloroethamine. Further, we conclude that same degree of beneficial effect on neutropenia can be achieved by starting G-CSF therapy 1, 3, 5, or 7 days after mechloroethamine. However, this data is based on studies with one chemotherapeutic agent administered for only one cycle and quite different results may be attained with a more myeloablative drug or after multiple cycles of therapy.

ACKNOWLEDGMENT

We express our sincere appreciation to Mike Clark for his dedication to the project and continued technical expertise and to Drs. R. Riddway and D. Gardner for their veterinary support.

REFERENCES

3. Souza LM, Boone TC, Gabrilove JL, Lai PH, Zsebo KM,


A comparison of therapeutic schedules for administering granulocyte colony-stimulating factor to nonhuman primates after high-dose chemotherapy

BR Meisenberg, TA Davis, AJ Melaragno, R Stead and RL Monroy