Acceleration of Hematopoietic Reconstitution With a Synthetic Cytokine (SC-55494) After Radiation-Induced Bone Marrow Aplasia

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The synthetic cytokine (Synthokine) SC-55494 is a high-affinity interleukin-3 (IL-3) receptor ligand that stimulates greater in vitro multilineage hematopoietic activity than native IL-3, while inducing no significant increase in inflammatory activity relative to native IL-3. The aim of this study was to investigate the in vivo hematopoietic response of rhesus monkeys receiving Synthokine after radiation-induced marrow aplasia. Administration schedule and dose of Synthokine were evaluated. All animals were total-body irradiated (TBI) with 700 cGy Co gamma radiation on day 0. Beginning on day 1, cohorts of animals (n = 5) received Synthokine subcutaneously (SC) twice daily with 25 μg/kg/d or 100 μg/kg/d for 23 days or 100 μg/kg/d for 14 days. Control animals (n = 9) received human serum albumin SC once daily at 15 μg/kg/d for 23 days. Complete blood counts were monitored for 60 days postirradiation and the durations of neutropenia (NEUT; absolute neutrophil count (ANC) <500/μL) and thrombocytopenia (THROM; platelet count <20,000/μL) were assessed. Synthokine significantly (P < .05) reduced the duration of THROM versus the HSA-treated animals regardless of dose or protocol length. The most striking reduction was obtained in the animals receiving 100 μg/kg/d for 23 days (THROM = 3.5 ± 1.25 days in HSA control animals). Although the duration of NEUT was not significantly altered, the depth of the nadir was significantly lessened in all animal cohorts treated with Synthokine regardless of dose versus schedule length. Bone marrow progenitor cell cultures indicated a beneficial effect of Synthokine on the recovery of granulocyte-macrophage colony-forming units that was significantly higher at day 24 post-TBI in both cohorts treated at 25 and 100 μg/kg/d for 23 days relative to the control animals. Plasma pharmacokinetic parameters were evaluated in both normal and irradiated animals. Pharmacokinetic analysis performed in irradiated animals after 1 week of treatment suggests an effect of repetitive Synthokine schedule and/or TBI on distribution and/or elimination of Synthokine. These data show that the Synthokine, SC55494, administered therapeutically post-TBI, significantly enhanced platelet recovery and modulated neutrophil nadir and may be clinically useful in the treatment of the myeloablated host.

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INTERLEUKIN-3 (IL-3) or multi–colony-stimulating factor (CSF) is a pivotal T-cell–derived cytokine in the stimulation of hematopoiesis. In vitro studies have shown that IL-3 stimulated the proliferation of early multipotential progenitors as well as the growth and differentiation of lineage-restricted colony-forming cells. Thus, IL-3 has been reported to stimulate both megakaryocyte progenitor cells and megakaryocytes. Variable responses to native IL-3 in normal monkeys predicted an equivocal response in preclinical and clinical evaluations in situations of marrow failure syndromes and radiation or chemotherapy-induced marrow aplasia. Gillio et al showed that, although IL-3 enhanced myeloid recovery in cyclophosphamide- or 5-fluorouracil (FU)–treated nonhuman primates, platelet recovery could not be shown. In contrast, it has been shown that IL-3 enhanced regeneration of platelets and reduced the duration of thrombocytopenia while showing no effect on recovery of neutrophils in a nonhuman primate model of radiation-induced marrow aplasia. Recently, Winton et al showed that once daily administration of IL-3 in a nonhuman primate model of hepsulfam-induced pancytopenia had no demonstrable effect on regeneration of platelets or neutrophils. Clinical studies using IL-3 in marrow failure syndromes or after chemotherapy with or without bone marrow transplantation have shown modest but variable activity in accelerating hematopoietic recovery. Therefore, despite its therapeutic potential, native IL-3 is characterized by a relatively narrow therapeutic index due to the intrinsic inflammatory activity of this cytokine. The structure activity relationships of IL-3 have been explored to perform rational design of clinically useful derivatives that would enhance the therapeutic index by reducing hematopoietic cytopenia as well as associated toxicity. The synthetic cytokine, Synthokine (SC-55494), is a high-affinity IL-3 receptor ligand that has shown 10- to 20-fold greater biologic activity than native IL-3 in human hematopoietic cell proliferation and marrow colony-forming unit assays. Synthokine priming of normal human peripheral blood leukocytes only induced a twofold increase in histamine release and sulfidoleukotriene (LTC4) synthesis versus human IL-3. The functional selectivity of Synthokine may be attributed to the greater binding affinity for the α/β IL-3 receptor complex that Synthokine exhibited relative to native IL-3. The aim of this study was to investigate the therapeutic efficacy of Synthokine on hematopoietic reconstitution in a nonhuman primate model of high-dose, radiation-induced marrow aplasia.

MATERIALS AND METHODS

Animals

Domestic-born male rhesus monkeys, Macaca mulatta, with a mean weight 3.9 ± 0.2 kg, were housed in individual stainless steel cages.
cages in conventional holding rooms at the Armed Forces Radiobiology Research Institute (AFRRI) in an animal facility accredited by the American Association for Accreditation of Laboratory Animal Care. Monkeys were provided with 10 air changes per hour of 100% fresh air, conditioned to 72°F ± 2°F with a relative humidity of 50% ± 20% and were maintained on a 12-hour light/dark full spectrum light cycle, with no twilight. Monkeys were provided with commercial primate chow, supplemented with fresh fruit and tap water ad libitum. Research was conducted according to the principles enunciated in the Guide for the Care and Use of Laboratory Animals, prepared by the Institute of Laboratory Animal Resources, National Research Council.

Irradiation

Monkeys placed in an Lucite restraining chair, after a prehabituation period, were bilaterally, total-body irradiated (TBI) with cobalt-60 gamma radiation at the AFRRI Cobalt-60 Facility to a total midline dose of 700 cGy at a dose rate of 40 cGy/min. Dosimetry was performed using a paired 0.5-ml tissue equivalent ionization chamber, whose calibration was traceable to the National Institute of Standards and Technology.

Recombinant Cytokines

Synthokine, SC-55494, is an IL-3 receptor agonist consisting of 112 amino acids. This Escherichia coli-derived protein has 21 amino acids deleted from the N- and C-terminal regions, in addition to 27 amino acid position changes relative to native IL-3.19 The Synthokine or the control protein (human serum albumin [HSA]; Miles Inc, Cutter Biological, Elkhart, IN) was administered as a 1-ml bolus subcutaneous (SC) injection.

Radiation Study Design

The animals were randomly assigned to one of four treatment groups composed of a minimum of five animals. Each animal was irradiated on day 0. On day 1, groups of animals received Synthokine (100 μg/kg/d, SC, twice daily [BID] for 14 consecutive days or 100 μg/kg/d or 25 μg/kg/d, SC, BID for 23 consecutive days) or HSA (15 μg/kg/d, SC, every day [QD] for 23 consecutive days).

Pharmacokinetic Study Design

Normal rhesus monkeys (n = 2/group) received 25 μg/kg intravenous (IV) bolus doses of either Synthokine or native human IL-3 via a saphenous or cephalic vein, and plasma samples were collected at intervals over a 5-hour time frame. A second pharmacokinetic study was performed on animals in the therapeutic study before (day −6) and day 7 postirradiation. These animals (n = 2) received 25 μg/kg, SC bolus doses of Synthokine, and plasma samples were collected at intervals over an 8-hour time frame.

Clinical Support

An antibiotic regimen was initiated prophylactically when the white blood cell count (WBC) was less than 1,000/μL and continued daily until the WBC was greater than 1,000/μL for 3 consecutive days. Gentamicin (Lypphenon, Deerfield, IL; 10 mg/d, SC, QD) and rocephin (Roche, Nutley, NJ; 250 mg/d, SC, QD) were administered. Fresh, irradiated (1,500 cGy 60Co gamma radiation) whole blood (approximately 30 mL/ transfusion) from a random donor pool (monkeys of >10 kg body weight) was administered when the PLT count was less than 20,000/μL and the hematocrit was less than 18%. Transfusions and antibiotics were required to ensure 100% survival in HSA-treated animals (unpublished results).

Hematologic Evaluations

Peripheral blood. Peripheral blood was obtained from the saphenous vein to assay complete blood (Model S Plus II; Coulter Electronics, Hialeah, FL) and differential counts (Wright-Giemsa Stain; Ames Automated Slide Stainer, Elkhart, IN). Baseline levels (BL) were obtained before irradiation. These parameters were monitored for 60 days after irradiation and the durations of neutropenia (absolute neutrophil count [ANC] <500/μL) and thrombocytopenia (platelet [PLT] count <20,000/μL) were assessed. Whole blood transfusions could have possibly altered the ANC and PLT count; therefore, when determining the durations of neutropenia and thrombocytopenia, ANC and PLT counts had to be maintained for 3 consecutive days above threshold levels after the first increase for a true recovery to be noted.

Bone marrow. Approximately 2 ml of bone marrow was aspirated from the humerus and/or iliac crest of anesthetized primates (ketamine, 10 mg/kg administered intramuscularly with a 21-gauge needle in 0.3 ml volume) into preservative-free heparinized syringes. Low-density (<1.077 g/mL) mononuclear cells (MNCs) were separated using Histopaque (Sigma, St Louis, MO) and resuspended in Iscove's modified Dulbecco's medium (IMDM; GIBCO, Grand Island, NY). The clonogenicity of bone marrow progenitor cells was assessed in short-term culture assay. Culture medium contained 0.9% methylcellulose (MethoCult H4230; Stem Cell Tech, Vancouver, British Columbia, Canada) in IMDM with 30% fetal calf serum (HyClone Laboratories, Logan, UT). In addition, a combination of recombinant human cytokines (granulocyte colony-stimulating factor [G-CSF] at 5 ng/ml; Amgen, Thousand Oaks, CA), mast cell growth factor (MGF; 50 ng/ml; Immunex Corp. Seattle, WA), erythropoietin (EPO; 2 U/mL; Behringwerke, Marburg, Germany), IL-3 (20 ng/ml), granulocyte-macrophage colony-stimulating factor (GM-CSF; 5 ng/ml), and IL-6 (40 ng/mL; Sandoz Pharmaceuticals, East Hanover, NJ), and transferrin (10 μg/mL; Sigma) were added. MNCs were cultured at a plating density of 5 × 10^6 cells/ml (days 0, 24, and 46 postirradiation) or 1 × 10^7 cells/ml (day 16 postirradiation). Cells were incubated for 10 days at 37°C with 5% CO₂ in air in a fully humidified incubator. Granulocyte-macrophage colony-forming unit (GM-CFU) and burst-forming unit-erythroid (BFUe) derived colonies (>50 cells) were separated using Histopaque (Sigma, St Louis, MO), and resuspended in IMDM. The clonogenicity of bone marrow progenitor cells was assessed in short-term culture assay. Culture medium contained 0.9% methylcellulose (MethoCult H4230; Stem Cell Tech, Vancouver, British Columbia, Canada) in IMDM with 30% fetal calf serum (HyClone Laboratories, Logan, UT). In addition, a combination of recombinant human cytokines (granulocyte colony-stimulating factor [G-CSF] at 5 ng/ml; Amgen, Thousand Oaks, CA), mast cell growth factor (MGF; 50 ng/ml; Immunex Corp. Seattle, WA), erythropoietin (EPO; 2 U/mL; Behringwerke, Marburg, Germany), IL-3 (20 ng/ml), granulocyte-macrophage colony-stimulating factor (GM-CSF; 5 ng/ml), and IL-6 (40 ng/mL; Sandoz Pharmaceuticals, East Hanover, NJ), and transferrin (10 μg/mL; Sigma) were added. MNCs were cultured at a plating density of 5 × 10^6 cells/ml (days 0, 24, and 46 postirradiation) or 1 × 10^7 cells/ml (day 16 postirradiation). Cells were incubated for 10 days at 37°C with 5% CO₂ in air in a fully humidified incubator. Granulocyte-macrophage colony-forming unit (GM-CFU) and burst-forming unit-erythroid (BFUe) derived colonies (>50 cells) were expressed as the number of CFU/10^5 MNCs.

Pharmacokinetic Analysis

Affinity-purified goat anti-Synthokine antibody and affinity-purified goat antinative human IL-3 antibody were used in sandwich enzyme-linked immunosorbent assays (ELISAs) to determine Synthokine and native human IL-3 plasma levels. Synthokine or native human IL-3 in plasma was bound in microtiter plate wells precoated with the appropriate goat antibody. The plates were washed, incubated with additional goat antibody conjugated with peroxidase, washed again, and allowed to react with peroxidase substrate and the optical density (OD) of the resulting peroxidase product was determined. The plasma determinations were performed using nonvalidated assays with standard curve range lower limits of 1 ng/mL.

Detection of Rhesus Monkey-anti-Synthokine Ig

Antibody titers in plasma samples from Rhesus monkeys dosed with 100 μg/kg BID Synthokine were determined by capture ELISA. Samples were tested from days 0; 6, 7, 8; or 12 or 14; 15; 16; and 29, 30, or 31. Ninety-six-well microtiter plates (Dynatech-Immunon II, Chantilly, VA) were coated with goat-anti-Synthokine affinity-purified polyclonal antibody. Each well received 150 μl of 100 mmol/L NaHCO₃ (Fisher Scientific, Pittsburgh, PA), pH 8.2, containing 5 μg/mL goat-anti-Synthokine polyclonal antibody. Plates
were incubated overnight at room temperature in a chamber maintaining 100% humidity. The following morning, wells were emptied and remaining reactive binding sites on the plastic surface of each were well blocked for 1 to 1.5 hours at 37°C (Precision Gravity Convection Incubator, Chicago, IL) in 100% humidity with 200 μL of 10 mmol/L phosphate-buffered saline (PBS; GIBCO), pH 7.4, containing 3% bovine serum albumin (BSA; Sigma) and 0.5% polyoxyethylene-sorbitan monolaurate (Tween 20; Sigma). Wells were emptied and washed by filling each well four times with 150 mmol/L NaCl containing 0.05% Tween 20 (wash buffer). For each sample evaluated, one set of duplicate wells received 150 μL of dilution buffer (10 mmol/L PBS, pH 7.4, 0.1% Tween 20) containing 1 μg/mL of pharmaceutical grade recombinant human G-CSF (rhG-CSF; Amgen) as a control. A second set of duplicate wells received 150 μL of dilution buffer containing 1 μg/mL Synthokine. Plates were incubated for 2.5 hours in a humidified chamber at 37°C. Wells were emptied and each well was washed four times with wash buffer. Dilutions of plasma samples were then added to their respective plates. Rows A through G of each plate received 150 μL of dilution buffer. Row H received 200 μL of plasma diluted 1:100. Fifty microliters was removed from row H and iterated 1:4 through row A in a serial fashion to cover a dilution range from 1:100 to 1:1,638,400. Plates were incubated for 1.5 hours in a humidified chamber at 37°C. Plates were emptied and each well was washed four times with wash buffer. Each well received 150 μL of dilution buffer containing 0.1 μg/mL of peroxidase-conjugated goat--anti-rhesus monkey Ig (Nordic Immunologicals, Capistrano Beach, CA). Plates were incubated for 1.5 to 2 hours at 37°C in a humidified chamber. Plates were emptied and each well was washed four times with wash buffer. Each well received 150 μL of TMB peroxidase substrate (Kirkegaard and Perry, Gaithersburg, MD). Plates were developed at room temperature for 10 minutes and read on an ELISA reader (Titertek; ICN Medical, Costa Mesa, CA) at a test wavelength of 650 mmol/L. Plasma antibody titers were calculated for each sample after subtracting the average OD of duplicate wells receiving rhG-CSF plus plasma from the corresponding average OD of duplicate wells receiving Synthokine plus plasma.

Statistical Analysis

The Normal Scores Test was used to make pairwise comparisons of the durations of neutropenia and thrombocytopenia. The test was performed using the software package StatXact (Cytel Software Corp. Cambridge, MA). The Mann-Whitney test was used to evaluate the statistical significance between the medians. Bone marrow-derived clonogenic activities were analyzed by two methods. Comparison of treatment days post-TBI to a historical baseline were made as a one-sided randomization t-test and a Bonferroni correction factor of 3 was applied to the P values. The comparison of treated groups versus the time-matched, HSA-treated controls were made using the Dunnett’s Test. A log transformation was used to correct for variance differences. These tests were performed using the software package SYSTAT (SYSTAT, Inc, Evanston, IL).

RESULTS

Pharmacokinetics of Synthokine in Normal and Irradiated Monkeys

The results indicated similar pharmacokinetic behavior of Synthokine and native IL-3 after IV injection in the rhesus monkey. The half-life was somewhat shorter (\(T_{1/2} = 46.1\) minutes vs 52.5 minutes), but clearance was slower (4.38 mL/min/kg vs 5.51 mL/min/kg) for Synthokine compared with native IL-3 (Table 1).

The bioavailability of Synthokine administered SC in the rhesus monkey in this study cannot be calculated directly because the IV and SC doses were administered to separate groups of monkeys. However, comparison of the IV and SC area under the curve (AUC) values indicates a bioavailability of approximately 100%. Synthokine was detected in the plasma collected from 0.25 to 8.0 hours after SC administration of 25 μg/kg/dose. The average CMAX in normal monkeys was 25.7 ng/mL, whereas the average TMAX and AUC were 1.3 hour and 106 ng/mL/h (0- to 8-hour period; Table 1).

Plasma concentrations after SC administration to irradiated animals (measured at day 7 post-TBI) were between two and three times higher than values determined in the same animals before irradiation (Table 2). The average CMAX on day 7 after TBI was 55.9 ng/mL, whereas the average TMAX and AUC were 1.8 hour and 285 ng/mL/h, respectively, relative to values for CMAX of 25.7 ng/mL, TMAX of 1.3 hour, and AUC of 106 ng/mL/h for Synthokine administered to animals before radiation exposure.

ELISA Determination of Rhesus Monkey—Anti-Synthokine Plasma Ig

There were no antibody titers to Synthokine detected at any evaluated time points post-TBI in the animals receiving 100 μg/kg/d for either the 14-day or 23-day schedule.

Modulation of Thrombocytopenia

Thrombocytopenia (PLT <20,000/μL) was evident in HSA-treated control animals for an average of 12.5 days (days 10 through 22) consequent to 700 cGy TBI (Fig 1A

### Table 1. Plasma Pharmacokinetic Parameters for Synthokine and Native IL-3 After Intravenous Administration of 25 μg/kg to Rhesus Monkeys

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters*</th>
<th>Synthokine</th>
<th>Native IL-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elimination half-life (min)</td>
<td>46.1</td>
<td>52.5</td>
</tr>
<tr>
<td>Clearance (mL/min/kg)</td>
<td>4.38</td>
<td>5.51</td>
</tr>
<tr>
<td>Vₐ (L/kg)</td>
<td>0.058</td>
<td>0.078</td>
</tr>
<tr>
<td>Vₐmax (L/kg)</td>
<td>0.282</td>
<td>0.408</td>
</tr>
<tr>
<td>AUC (ng/mL/hr) (0-∞)</td>
<td>95.6</td>
<td>77.6</td>
</tr>
</tbody>
</table>

* Average values for three monkeys.

### Table 2. Plasma Pharmacokinetic Parameters for Synthokine (SC-5545) After SC Administration of 25 μg/kg to Rhesus Monkeys

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters*</th>
<th>Day -6</th>
<th>Day +7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (μg/kg)</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>25.7</td>
<td>55.9</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>1.3</td>
<td>1.8</td>
</tr>
<tr>
<td>AUC (ng/mL/h) (0-8 h)</td>
<td>106</td>
<td>285</td>
</tr>
</tbody>
</table>

* Average values for 2 monkeys. Monkey received TBI with 60Co gamma radiation to a total midline dose of 700 cGy on day 0. Each animal received a 25 μg/kg SC dose of Synthokine on day -6 (before TBI) and day +7 (after TBI). Each animal also received 25 μg/kg SC doses of Synthokine twice daily on days 1 through 6.
and Table 3). Administration of Synthokine at 25 or 100 \( \mu g/\) kg/d for 23 consecutive days post-TBI significantly reduced the duration of thrombocytopenia relative to HSA-treated controls to 7.4 days (\( P = .019 \)) and 3.5 days (\( P = .001 \)), respectively (Fig 1A and Table 3), whereas the 100 \( \mu g/\) kg/d dose administered for only 14 consecutive days post-TBI reduced the thrombocytopenic period to 6.2 days (\( P = .003 \); Fig 1B and Table 3). The administration of Synthokine for 23 days at the 100 \( \mu g/\) kg/d dosage produced a reduction in the duration of thrombocytopenia that was significantly greater than both the 14-day protocol at the same dose (3.5 v 6.2 days, \( P = .048 \)) and the same 23-day protocol at the lower dosage of 25 \( \mu g/\) kg/d (3.5 v 7.4 days, \( P = .028 \)). There was no significant (\( P = .187 \)) difference in the duration of thrombocytopenia when Synthokine was administered for 14 days at 100 \( \mu g/\) kg/d (6.2 days) versus 25 \( \mu g/\) kg/d dose for 23 days (7.4 days).

The administration of Synthokine, regardless of treatment protocol, accelerated platelet recovery, with preirradiation baseline levels being attained within approximately 28 days after exposure. In contrast, HSA-treated controls required approximately 40 days to attain baseline levels (Fig 1A). The platelet nadir was significantly decreased relative to that of the HSA-treated controls only in the cohort of animals treated with Synthokine at 100 \( \mu g/\) kg/d for 23 consecutive days.
Table 3. The Effect of Synthokine Administration on the Duration of Thrombocytopenia and Neutropenia in Rhesus Monkeys Irradiated at 700 cGy 60Co

<table>
<thead>
<tr>
<th>Synthokine</th>
<th>Thrombocytopenia</th>
<th>Neutropenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 µg/kg/d</td>
<td>3.5 d†</td>
<td>14.1 d</td>
</tr>
<tr>
<td>25 µg/kg/d</td>
<td>6.2 d*</td>
<td>15.7 d</td>
</tr>
<tr>
<td>15 µg/kg/d</td>
<td>7.4 d*</td>
<td>14.2 d</td>
</tr>
</tbody>
</table>

Monkeys whole body irradiated with 60Co gamma radiation were treated with control protein (HSA) or Synthokine according to protocol. Neutropenia is defined as an ANC less than 500/µL. Thrombocytopenia is defined as a platelet count of less than 20,000/µL.

* Statistically significant difference from the HSA-treated controls.
† Statistically significant difference from the 100 µg/kg/d, 14-day protocol and the 25 µg/kg/d, 23-day protocol.

Modulation of Red Blood Cells and Transfusion Requirements

Synthokine administration at 100 µg/kg/d for 23 days induced a significant increase (P < .05) in the nucleated red blood cell count (NRBC) between days 20 and 23 post-TBI versus the HSA-treated controls (Fig 2). The 14-day administration of Synthokine at 100 µg/kg/d significantly increased the NRBC between days 18 and 23 post-TBI. There was no evidence of clinical bleeding in any particular group of animals. All animals did not require transfusions. The HSA and 25 µg/kg/d Synthokine groups received an average of 1.5 transfusions per animal, whereas the two 100 µg/kg/d cohorts treated for 14 or 23 consecutive days received an average of less than one transfusion per animal (Table 4).

Modulation of Neutropenia

Although the duration of neutropenia (ANC <500/µL) was not significantly altered with Synthokine treatment (P > .50), the depth of neutrophil nadir was lessened in all cohorts of animals treated with Synthokine relative to the HSA-treated control animals (Fig 3A and B and Table 3). The mean duration of neutropenia was 14.9 days in HSA-treated animals versus 14.2 and 14.1 days in the 25 µg/kg/
and 100 μg/kg/d animals treated for 23 consecutive days and 15.7 days for the cohort treated with 100 μg/kg/d for only 14 consecutive days. Treatment with Synthokine did not accelerate recovery of neutrophils to preirradiation levels relative to the HSA-treated animals (Fig 3A, B) and did not modify the antibiotic requirements during the neutropenic period.

**Effect of Synthokine Administration on Bone Marrow Progenitor Cell Recovery**

The bone marrow-derived clonogenic activities for BFUe and GM-CFU were significantly (P < .05) depressed through day 46 post-TBI in the HSA-treated controls (Fig 4A and B). In contrast, the BFUe and GM-CFU values for all Synthokine treatment protocols recovered to BL levels no later than day 46 post-TBI (Fig 4A and B). The 23-day administration of Synthokine at either 25 or 100 μg/kg/d significantly increased (P < .05) the GM-CFU activity versus the time-matched, HSA-treated controls by day 24 post-TBI (Fig 4B). The animals receiving the shortened Synthokine protocol (14 days) also displayed increased GM-CFU activity at day 24 post-TBI versus the time-matched controls (P = .06); however, a significant increase (P = .03) was not noted until day 46 post-TBI (Fig 4B). The BFUe activity in all three Synthokine treatment groups was significantly (P < .05) increased above the time-matched, HSA-treated controls at day 46 post-TBI (Fig 4A).

**DISCUSSION**

Hematopoietic growth factors such as GM-CSF, IL-3, IL-6, IL-11, leukemia inhibitory factor (LIF), and megakaryo-
cyte growth and development factor (MGDF) or thrombopoietin (Tpo) have been shown to stimulate megakaryocyte differentiation in vitro and enhance platelet production in preclinical models of normal and radiation or drug-induced marrow aplasia. The lineage-specific cytokines G-CSF and GM-CSF have shown therapeutic efficacy in accelerating production of neutrophils. Thrombopoietic efficacy in clinical situations of marrow failure has not been realized for GM-CSF, whereas IL-3 and IL-6 efficacy has been questionable due to their low therapeutic indices and IL-11 is currently in clinical trials.

The demonstrated in vitro efficacy of IL-3 as a growth factor for stimulation of multipotent and committed myeloid progenitors suggested that it may play a major role as a primer by increasing the action of more lineage-restricted cytokines such as G-CSF, GM-CSF, IL-6, and IL-11. Such efficacy has recently been shown in preclinical models of radiation or drug-induced marrow apasia for the combination of IL-3 with GM-CSF or IL-6 and forecast the efficacy of the IL-3/GM-CSF fusion protein, PIXY321. In addition, IL-3 has been shown to promote survival of primitive murine and human hematopoietic progenitor cells. Brandt et al recently showed that addition of IL-3 to cultures of highly enriched human committed progenitor cells delayed the appearance of morphologic changes and DNA fragmentation patterns associated with apoptosis. The demonstrated efficacy of IL-3 in promoting increased proliferation as well as survival of hematopoietic progenitor cells warranted further analysis of the growth factor with regard to structure function relationships and improving its clinical therapeutic index.

The synthetic cytokine, Synthokine, is a high-affinity IL-3 receptor ligand that has shown greater in vitro multilineage growth factor activity than native IL-3 while inducing no significant increase of inflammatory activity. The enhanced binding to the α subunit has been implicated as a key determinant of Synthokine’s increased in vitro biologic activity. The purpose of this study was to evaluate the efficacy of Synthokine in decreasing the time to recovery from neutropenia and thrombocytopenia in a nonhuman primate model of radiation-induced marrow aplasia. The protocols evaluated two doses of Synthokine (25 vs 100 µg/kg/d) administered over 23 consecutive days postirradiation, as well as one dose (100 µg/kg/d) of Synthokine administered over both 14-day and 23-day schedules relative to HSA-treated control animals.

We showed that Synthokine was able to ameliorate the clinically significant, radiation-induced nadirs in ANC and PLT, as well as to significantly decrease the duration of thrombocytopenia without significant toxicity or morbidity relative to HSA-treated controls. Neither the dose of Synthokine nor the protocol schedule affected the duration of neutropenia or recovery of ANC to baseline, preirradiation values. This occurs despite the fact that Synthokine administration accelerated the postirradiation recovery of marrow-derived GM-CFU progenitors relative to the control response. This apparent discrepancy between clonogenic data and peripheral blood leukocyte counts could be explained...
by the fact that the enhanced bone marrow GM-CFU recovery was observed from day 24 post-TBI. It would take approximately 10 days for these progenitors to differentiate into mature leukocytes. At that time (day 30 to 31 postirradiation), the ANC appeared to be greater in the Synthokine-treated animals (100 μg/kg/d for 23 days) relative to the HSA group. IL-3–stimulated proliferation of primate and human marrow progenitors has been shown with administration schedules as short as 7 consecutive days, whereas longer treatment schedules were required to stimulate variable levels of circulating neutrophils and platelets. These results suggested that the increase in mature cell lineages is dependent on the interaction of IL-3–primed progenitors with lineage-specific cytokines. In two previous studies, we were unable to show the efficacy of native IL-3 in reducing neutropenic duration or recovery of ANC to preirradiation levels. In contrast, Gilrio et al. reported that IL-3 reduced both neutrophil nadir and duration of neutropenia in cyclophosphamide and 5-FU–treated cynomolgus primates, whereas no increase in platelet production could be shown. Winton et al., using hepsulfam-induced pancytopenia in rhesus primates, could not show therapeutic efficacy of native IL-3 for restoring platelets or neutrophils. However, the administration protocol (a single daily, SC injection) may account for the ineffectiveness of the native IL-3 in this model. It is of interest that our pharmacokinetic analysis of Synthokine versus native IL-3 showed similar values and suggests a BID protocol for effective SC administration. It is also of interest that plasma concentrations of Synthokine were between two to three times higher at day 7 after TBI compared with their preirradiation values. These results indicate an effect of repetitive Synthokine administration (7 consecutive days) and/or of TBI on distribution and/or elimination of Synthokine.

Administration of Synthokine significantly enhanced the recovery of PLT to preirradiation levels and reduced the duration of thrombocytopenia at both doses and schedules used, as well as reduced the nadir of PLT at the highest dose administered over the 23-day schedule compared with the HSA-treated controls. We previously showed the efficacy of native IL-3 in accelerating PLT recovery and reducing the thrombocytopenic period. Two doses (25 and 100 μg/kg/d) of Synthokine were evaluated in this model because of noted modest hematopoietic activity of native IL-3 and Synthokine in rhesus primates bone marrow–derived cell cultures (attributable to 81% and 64% respective homology to rhesus IL-3; unpublished results). van Gils et al. have also shown that removal of IL-3 or kit-ligand, which could interfere with hematopoietic recovery, would provide the desired effect of enhancing hematopoietic production of both platelets and neutrophils. The demonstrated therapeutic efficacy of combined, concurrent native human IL-3 and GM-CSF as well as the IL-3/GM-CSF fusion protein PXY321 in a similar model of radiation-induced marrow aplasia would forecast efficacy for Synthokine and G-CSF.

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