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ORIGINAL ARTICLE

G.J. Nohynek · J.P. Plard · M.Y. Wells · A. Zerial
F. Roquet

Comparison of the potency of glycosylated and nonglycosylated recombinant human granulocyte colony-stimulating factors in neutropenic and nonneutropenic CD rats

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Abstract Recent studies in human bone-marrow culture and healthy human volunteers suggest that lenograstim [glycosylated, recombinant human granulocyte colony-stimulating factor (rHuG-CSF) produced in Chinese hamster ovary (CHO) cells] has greater in vivo potency than filgrastim [nonglycosylated, methionine-extended recombinant human granulocyte colony-stimulating factor (r-metHuG-CSF) produced in *Escherichia coli*]. To confirm and extend these results we investigated the in vivo potency of both products in normal rats and neutropenic CD rats as an animal model of chemotherapy-induced neutropenia. In normal rats, groups of eight normal male CD rats received four subcutaneous doses of 10, 30, or 100 μg/kg filgrastim or lenograstim on days 1–4 of the study, whereas a control group received the vehicle. Blood samples were collected from each animal before treatment (day −5) and on days 2, 3, 5, 8, and 12 of the study for determination of red blood cell (RBC), platelet, white blood cell (WBC), and differential counts. rHuG-CSF and r-metHuG-CSF produced increased WBC counts, principally due to elevated absolute neutrophil counts (ANCs); on days 2, 3, and 5, all groups receiving rG-CSF had ANCs that increased in a progressive and dose-related manner. With the exception of a single value, mean ANCs obtained on days 2, 3, and 5 in lenograstim-treated groups were higher (statistically significant on day 3 at 30 and 100 μg/kg and on day 5 at 10, 30, and 100 μg/kg) than the respective values obtained in filgrastim-treated groups. No compound-related effect was noted in RBC or platelet parameters. Neutropenia was induced in male CD rats (12 animals/group) with a single intraperitoneal dose of 50 mg/kg cyclophosphamide (CPA) on day 0. On days 1–4, CPA-treated groups were treated with the vehicle (control) or with filgrastim or lenograstim at 30 or 100 μg/kg per day. An additional group was not treated with CPA and served as the absolute control group. Blood was collected from alternating subgroups on study day −5 (pretest) and on days 2, 3, 4, 5, 6, 8, 9, and 12 for determination of RBC, platelet, WBC, and differential counts. No major adverse in-life effect was noted in neutropenic rats. Maximal depression of WBCs and ANCs occurred on day 5, followed by recovery to normal values by days 9 (ANC) and 12 (WBC). On day 3 and days 5–9, rHuG-CSF- and metHuG-CSF-treated groups had marked and dose-related increases in WBCs as compared with CPA-treated controls, principally due to elevated ANCs. With the exception of a few values, mean ANC values obtained in lenograstim-treated groups were consistently higher than the respective values obtained in filgrastim-treated groups; the difference was statistically significant on day 3 (30-μg/kg groups) and on days 6 and 8 (100-μg/kg groups). In conclusion, treatment of normal and neutropenic CD rats with lenograstim resulted in a dose-related elevation of ANCs that was consistently and significantly higher than the response to identical doses of filgrastim. These results suggest that lenograstim, the glycosylated form of rG-CSF, has superior in vivo potency in normal and neutropenic animals as compared with filgrastim, the nonglycosylated form of rG-CSF.

Key words rHuG-CSF · CD rats

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G.J. Nohynek (✉) · J.P. Plard · M.Y. Wells · A. Zerial · F. Roquet
Rhone-Poulenc Rorer, Drug Safety Department,
Centre de Recherche de Vitry-Alfortville, F-94403 Vitry-sur-Seine, France
Fax: +33-1-45738094.

Introduction

Neutrophils play a significant role in the host defense system of mammalian organisms. Production of neutrophils is maintained by hematopoietic stem cells in the bone marrow and is regulated by various cytokines, the most prominent of which is granulocyte colony-stimulating fac-

tor (G-CSF) [1]. G-CSF selectively promotes the proliferation, differentiation, and maturation of the neutrophil cell line; stimulates the release of mature neutrophils from hematopoietic tissues; and increases their functional activity [1, 2]. Human G-CSF is a peptide consisting of 177 amino acids; it has an approximate molecular weight of 20 kDa and is O-glycosylated on Thr 133. The carbohydrate chain on Thr 133 consists of D-galactose, *N*-acetylgalactosamine, and *N*-acetyl-neuraminic acid [3].

Chemotherapy or radiotherapy frequently induces myelosuppression, which may result in life-threatening infections secondary to neutropenia [4]; administration of G-CSF to neutropenic patients stimulates the production of neutrophils, shortens the duration of neutropenia following myelosuppression after chemotherapy or radiation exposure, and thereby reduces the risk of infection [5, 14]. Two preparations of recombinant human G-GSF are currently commercially available for clinical use: filgrastim, a nonglycosylated, methionine-extended *Escherichia coli*-derived form (r-metHuG-CSF) [6], and lenograstim, a glycosylated form (rHuG-CSF) that is derived from Chinese hamster ovary cells [7, 18]. rG-CSF is given to humans either subcutaneously or via intravenous infusion at a dose range of 2–10 μg/kg per day, depending on the type used and the clinical indication.

Although glycosylation does not appear to be essential for the biological activity of rG-CSF, it has been reported to improve rG-CSF's in vitro stability at pH values of 7–8, its thermostability, and its resistance to degradation by proteases [8, 9]. Recent results of human bone-marrow culture assays [10] and of a comparative study on peripheral blood progenitor-cell mobilization in healthy volunteers [20] indicate that lenograstim has a greater biological potency than filgrastim. However, given the considerable variability of individual responses in patients treated with rG-CSF [11, 15], it is not clear whether a significant difference in the mobilization of neutrophils exists between filgrastim and lenograstim under conditions of clinical use.

Our laboratory has accumulated a considerable historical data base on hematology parameters of CD rats. Given that this strain has relatively stable and homogeneous hematology values and responds readily to subcutaneous treatment with rG-CSF [17, 21], we compared the potency of glycosylated and nonglycosylated rG-CSF preparations using normal and neutropenic (cyclophosphamide pretreated) CD rats. The objective of our study was to evaluate whether a difference in potency exists between the two preparations of rG-CSF when these are studied in vivo.

Materials and methods

Dose levels and dosage.

Since rats appear to be less sensitive than humans to human rG-CSF [21], we selected a dose regimen of four subsequent daily subcutaneous doses and a dose range of 10–100 μg/kg rG-CSF. In addition, it had been shown that this dose range and regimen produced a marked dose response in terms of the degree and duration of stimulation of neutrophil production [12]. Leukopenia/neutropenia was induced by intraperitoneal administration of a single dose of 50 mg/kg cyclophosphamide (CPA) as based on the results of a preliminary investigation and published data, which indicated that this dose level produced in male CD rats marked leukopenia/neutropenia of a severity comparable with WHO grade III neutropenia in humans for several days [19, 21].

Compound/treatment solutions.

Commercial lenograstim (rHuG-CSF, Granocyte; batch number CB 05919) was provided by Chugai-Rhone-Poulenc (Antony, France) in vials containing 263 μg lyophilized rHuG-CSF, 50 mg mannitol, 0.1 mg polysorbate 20, and phosphate buffer (pH 6.5). The lyophilized compound was dissolved in 1 ml sterile water, resulting in a stock solution containing 263 mg rHuG-CSF/ml. For subcutaneous administration, 0.9 ml of this stock solution was diluted with 0.9% aqueous NaCl containing 0.1% human serum albumin to achieve a final volume of 15.8 ml containing 15 mg rHuG-CSF/ml. Commercial filgrastim (r-metHuG-CSF, Neupogen; batch number B 0624MFD0694) was obtained commercially in glass vials containing 1.6 ml of a 300-mg/ml solution of rHuG-CSF. For subcutaneous administration, 0.9 ml of this solution was diluted with aqueous 0.9% NaCl containing 0.1% human serum albumin to achieve a volume of 18 ml containing 15 mg r-metHuG-CSF/ml. Control groups received the vehicle, 0.9% aqueous NaCl containing 0.1% human serum albumin. Lyophilized CPA (Endoxan, 500 mg; lot number 591) was received from Laboratoire ASTA (Merignac, France) and was dissolved in 25 ml water for injection, resulting in a solution containing 20 mg CPA/ml; 2.5 ml/kg of this solution (50 mg CPA/kg body weight) was given intraperitoneally.

Animals and treatments.

A total of 128 male Sprague-Dawley-derived CD rats aged 6–7 weeks and weighing 160–180 g at study initiation were received from Charles River France (St. Aubin lès Elbeuf, France). Following an 11-day acclimatization period the animals were randomly assigned to 7 groups containing 8 animals each (study in normal rats) and 6 additional groups containing 12 animals each (study in neutropenic rats). The animals were individually identified by ear tattoo and were housed at four per cage in stainless steel cages under environmentally controlled conditions with free access to commercial rodent feed (UAR A04; Société UAR, Epinay sur Orge, France) and filtered tap water. Housing and animal care were in accordance with current guidelines issued by the European Community or the United States National Institute of Health.

In the study on normal rats the groups received four single daily sterile subcutaneous treatments (days 1–4) of 0 (vehicle control: aqueous 0.9% NaCl/0.1% human serum albumin), 10, 30, or 100 μg/kg lenograstim or filgrastim. Doses calculated on a body-surface-area basis were approximately 66, 200, or 660 μg/m^2, respectively. The administration volumes were 0.67, 2.0, or 6.7 ml/kg, respectively. The control group received four daily subcutaneous 6.7-ml/kg doses of the vehicle, corresponding to the largest volume given to the treated groups.

In the study on neutropenic rats, 6 groups of 12 male CD rats were used. Five groups received a single intraperitoneal dose of 50 mg/kg CPA, whereas an absolute control group received 2.5 ml/kg 0.9% saline (day 0). Four of the CPA-treated groups received daily subcutaneous doses of 30 or 100 μg/kg filgrastim or lenograstim on days 1–4. The remaining CPA group received the vehicle (aqueous 0.9% NaCl/0.1% human serum albumin) and served as a positive control group. The absolute control group received no treatment on days 1–4.

Parameters evaluated.

During the pretest period (days −5 through −1) and after the treatment period (days 5–12), all animals were checked daily for general condition and potential clinical signs. During the treatment period the animals were checked prior to each treatment and at 1 and 4 h after compound administration. The body weight was recorded during the pretest period and on days 1, 5, and 12 of the study (non-neutropenic rats) or on days 1, 5, 9, and 12 (neutropenic rats).

Table 1 WBC counts obtained in normal male CD rats treated on days 1–4 with daily subcutaneous doses of 10, 30, or 100 μg/kg filgrastim or lenograstim

Study day/dose group	Pretest (day –5)	Day 2	Day 3	Day 5	Day 8	Day 12
0 (control)	13.7 ± 2.2	14.1 ± 2.7	13.8 ± 2.8	14.9 ± 2.8	13.6 ± 2.9	13.4 ± 2.9
Filgrastim 10 μg/kg	10.5 ± 2.7	15.4 ± 3.2	17.9 ± 4.5	16.4 ± 3.0	15.7 ± 3.5	13.7 ± 2.6
Filgrastim 30 μg/kg	11.8 ± 1.7	22.1 ± 3.7	20.2 ± 3.2	21.2 ± 5.7	14.9 ± 2.6	13.4 ± 2.2
Filgrastim 100 μg/kg	10.5 ± 2.0	23.6 ± 3.6	20.3 ± 3.2	24.2 ± 4.4	12.2 ± 1.5	11.6 ± 1.5
Lenograstim 10 μg/kg	11.5 ± 2.1	17.7 ± 2.3	15.7 ± 3.0	17.1 ± 1.9	12.6 ± 2.9	11.5 ± 2.2
Lenograstim 30 μg/kg	11.1 ± 2.0	21.0 ± 4.9	25.1 ± 5.5*	26.5 ± 6.3	16.3 ± 3.1	12.0 ± 1.7
Lenograstim 100 μg/kg	10.7 ± 2.0	23.4 ± 4.8	30.0 ± 8.2*	29.8 ± 6.7*	12.6 ± 2.8	11.3 ± 3.2

*$P < 0.05$ versus the respective means of the filgrastim-treated group
[a] Data represent mean values ± SD, expressed as numbers of cells $\times 10^3$/μl. Pretreatment (reference) range $7.1–16.9 \times 10^3$ cells/μl

Table 2 WBC counts obtained in male CD rats treated on day 0 with 50 mg/kg CPA followed by 4 subcutaneous doses on days 1–4 of filgrastim or lenograstim at 30 or 100 μg/kg per day[a]

Study day/dose group	Pretest[b]	Day 2	Day 3	Day 4	Day 5	Day 6	Day 8	Day 9	Day 12
Absolute controls	9.55 ± 1.6	9.23 ± 0.60	10.29 ± 0.78	9.65 ± 1.40	10.72 ± 1.54	10.26 ± 1.56	11.38 ± 2.12	9.51 ± 0.40	10.49 ± 2.53
CPA controls	9.36 ± 1.63	3.20 ± 1.67	2.08 ± 0.49	2.17 ± 1.62	1.54 ± 0.35	2.46 ± 1.95	2.94 ± 0.35	4.23 ± 2.38	8.90 ± 2.54
CPA/filgrastim 30 μg/kg	9.49 ± 1.32	2.84 ± 0.41	3.83 ± 0.83	1.78 ± 0.14	1.86 ± 0.47	4.84 ± 2.19	4.60 ± 1.34	6.38 ± 1.98	8.78 ± 1.02
CPA/filgrastim 100 μg/kg	9.63 ± 1.49	3.15 ± 0.35	5.19 ± 1.09	1.97 ± 0.29	3.27 ± 2.21	6.92 ± 2.59	4.89 ± 1.53	6.59 ± 1.40	8.18 ± 1.92
CPA/lenograstim 30 μg/kg	9.76 ± 1.32	2.74 ± 0.64	4.52 ± 1.00	1.61 ± 0.38	2.24 ± 0.47	4.14 ± 1.84	5.60 ± 1.12	6.83 ± 1.22	9.21 ± 1.93
CPA/lenograstim 100 μg/kg	9.96 ± 1.21	3.73 ± 1.01	5.10 ± 1.27	2.35 ± 0.13	2.94 ± 1.01	9.94 ± 2.12	5.64 ± 1.64	6.58 ± 1.58	8.49 ± 1.77

*$P < 0.05$ (compared to respective means of the filgrastim-treated group)
[a] Data represent mean values ± SD, expressed as numbers of cells $\times 10^3$/μl. Pretreatment (reference) range $7.1–13.79 \times 10^3$ cells/μl
[b] Pretest data are the calculated mean values ± SD obtained from pretreatment blood samples taken over a 2-day period

For determination of hematology parameters, blood samples (0.5 ml per sample and per animal) were collected from all groups from the orbital venous plexus using slight diethylether anesthesia. Samples from the non-neutropenic groups were taken during the pretest period (day −5) and on days 2, 3, 5, 8, and 12 of the study. Each group of the neutropenic rats was randomly subdivided into two subgroups of six animals for blood collection. Blood samples were collected from alternating subgroups during the pretest period (days −3 or −2) and on days 2, 3, 4, 5, 6, 8, 9, and 12 of the study. Red blood cell (RBC), hemoglobin, and hematocrit counts as well as mean corpuscular volumes, mean corpuscular hemoglobin volumes, mean corpuscular hemoglobin concentrations, white blood cell (WBC) counts, and differential WBC counts were evaluated using a Technicon H1 system (Bayer Diagnostic, Puteaux, France). The reference range for hematology values in the study on normal rats was the range of pretreatment values obtained on day −5. The reference range for hematology values in the study on neutropenic rats was the range of pretreatment values obtained on days −3 and −2. On day 12 of the study, following collection of the last blood sample, animals from both studies were anesthetized by carbon dioxide inhalation and killed by exsanguination. No postmortem evaluation was performed.

Statistical methods.

Mean values and standard deviations were calculated for body weight and hematology parameters for each sex and group. Statistical comparison of the group mean body weight for treated groups versus controls was performed using Dunett's *t*-test. Significance was assumed at $P < 0.05$. The significance of differences observed in mean WBC and neutrophil counts between groups receiving filgrastim or lenograstim was evaluated using Student's *t*-test, and significance was assumed at $P < 0.05$. The area under the curve for absolute neutrophil counts (ANCs) was determined using commercial PC software (GraphPad PRISM; GraphPad Software, Inc., San Diego, Calif., USA).

Results

In-life observations

Normal rats

As compared with the saline-treated control group, no clinical sign, behavioral change, or effect on body weight was observed in rG-CSF-treated groups.

Neutropenic rats

No clinical sign related to treatment was observed in neutropenic rats. Treatment-related effects were limited to a slight reduction in the mean body-weight gain (as compared with saline-treated controls) between days 1 and 5, which was noted in all groups receiving CPA. Body-weight development during days 5–12 was comparable in all groups.

White Blood Cell parameters

The group mean WBC counts are shown in Table 1. Both filgrastim and lenograstim caused a dose-related increase in the mean absolute number of WBCs counted on days 3 and

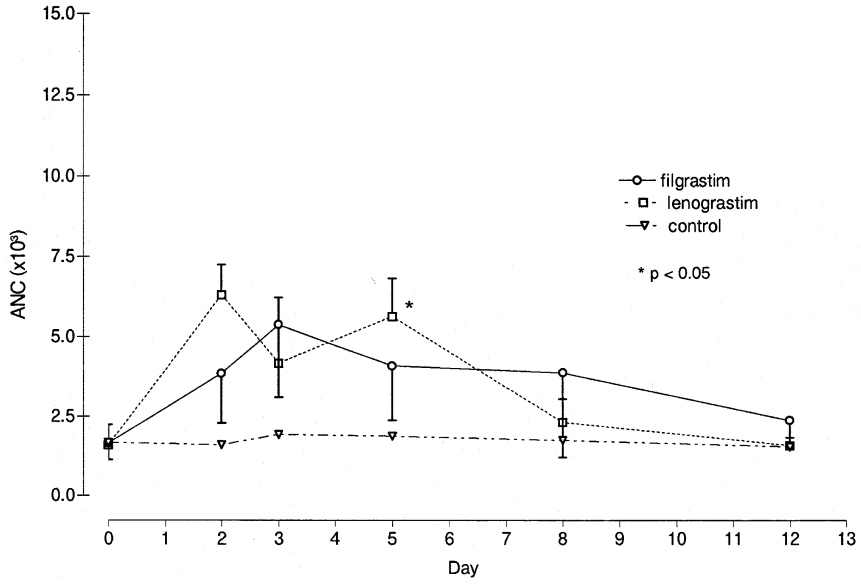

Fig. 1 Mean ANCs ($\times 10^3/\mu l$) obtained in normal male CD rats treated with four subcutaneous doses (days 1–4) of filgrastim or lenograstim at 10 μg/kg per day

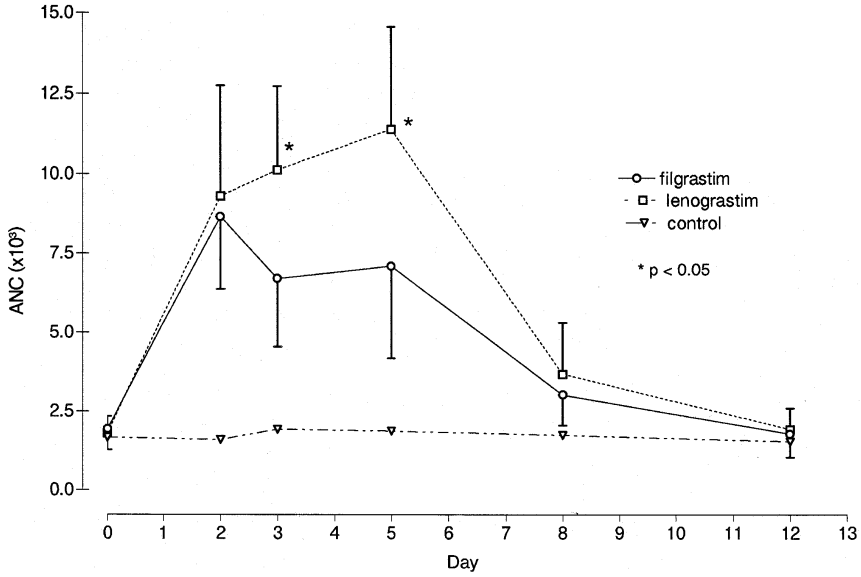

Fig. 2 Mean ANCs ($\times 10^3/\mu l$) obtained in normal male CD rats treated with four subcutaneous doses (days 1–4) of filgrastim or lenograstim at 30 μg/kg per day

5 of the study as compared with the mean control values. These increases were primarily due to increases in the absolute number of neutrophils in all dose groups.

The group mean WBC counts obtained in neutropenic rats are shown in Table 2. CPA treatment caused mild to marked decreases in the mean absolute number of WBCs counted as compared with the mean saline control values in all groups throughout the study (maximum −86% on day 5). The decreases in WBCs were primarily due to decreases in the absolute number of neutrophils and lymphocytes in all dose groups and were less severe in groups treated with filgrastim or lenograstim. The effect of filgrastim and lenograstim on WBC counts was principally due to increases in the absolute number of neutrophils in the groups treated with these compounds.

Neutrophils

Normal rats. The kinetics of the neutrophil response observed at 10, 30, and 100 μg/kg per day is displayed in Figs. 1–3. Lenograstim caused greater increases in neutrophil counts at all dose levels (maximum +8-fold ANC on day 5) than did filgrastim (maximum +4.7-fold on day 5), with the single exception being the 10-mg/kg dose of lenograstim on day 3. On days 3 and 5, 30 mg/kg lenograstim caused increases in ANC that were comparable with the increases caused by 100 mg/kg filgrastim. Neutrophil values for both compounds returned to the reference range by day 8 of the study. The calculated $AUC_{day\ 1-12}$ (area under the curve of mean the ANC above the saline control baseline versus time) was 55.6 (10^3 cells/μl days) for the group receiving lenograstim at 30 mg/kg per day, as

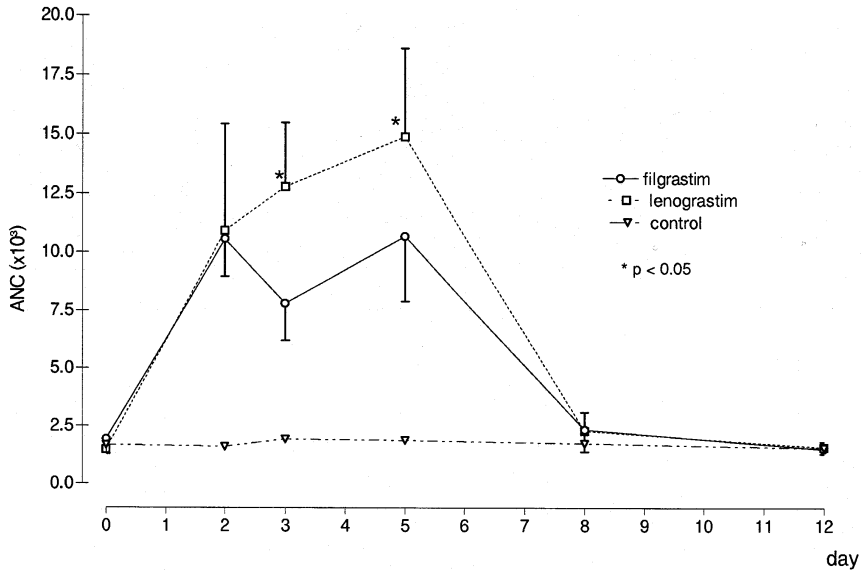

Fig. 3 Mean ANCs ($\times 10^3/\mu l$) obtained in normal male CD rats treated with four subcutaneous doses (days 1–4) of filgrastim or lenograstim at 100 μg/kg per day

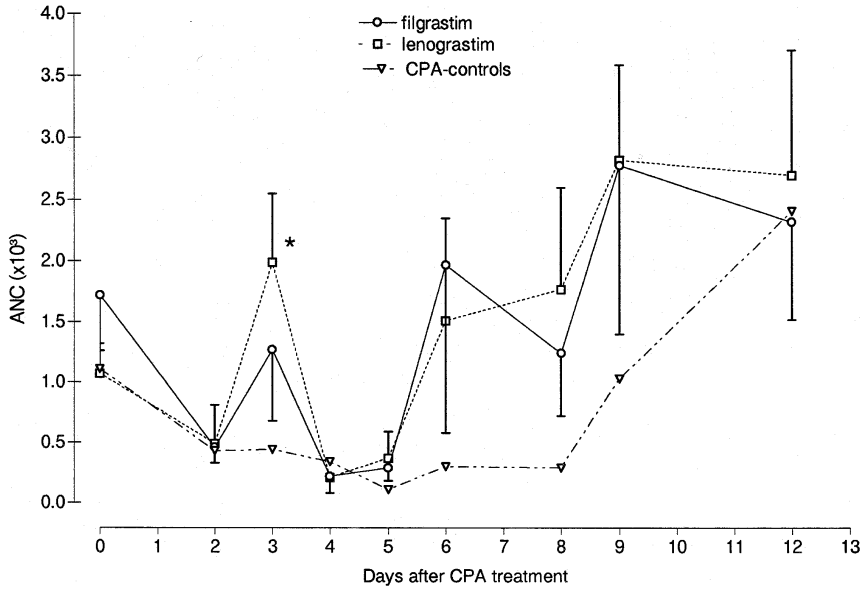

Fig. 4 Mean ANCs ($\times 10^3/\mu l$) obtained in male CD rats treated with 50 mg/kg CPA (day 0) followed by four subcutaneous doses (days 1–4) of filgrastim or lenograstim at 30 μg/kg per day. * $P < 0.05$

compared with a calculated AUC of 36.0 (10^3 cells/ml × days) for the group receiving an equal dose of filgrastim. The calculated $AUC_{day\ 1-12}$ was 64.6 (10^3cells/ml × days) for the group receiving lenograstim at 100 mg/kg per day as compared with 46.5 (10^3cells/ml × days) for the group receiving an equal dose of filgrastim.

Neutropenic rats. The kinetics of the neutrophil response observed at 30- and 100-μg/kg daily doses of filgrastim or lenograstim are shown in Figs. 4 and 5. The mean ANCs obtained in rats treated with CPA only were markedly decreased as compared with the mean saline control values at all sampling dates (maximum −91% on day 5), except on day 12, when the mean ANC was increased (+54% above the saline control mean). The mean ANCs obtained in groups treated with filgrastim or lenograstim were markedly increased on days 3, 6, and 9 and were minimally to moderately increased on days 8 (100 mg/kg lenograstim only) and 12 (all treated groups) as compared with the mean saline control values. Increases were dose-related in both the filgrastim-treated groups and the lenograstim-treated groups; in general, lenograstim caused greater increases than did filgrastim. The maximal increase (+3.7-fold) was observed in the 100-mg/kg lenograstim group on day 6.

When groups treated with filgrastim or lenograstim were compared with the group given CPA only, a clear dose-related increase in ANCs was observed with both compounds. With few exceptions the mean ANCs recorded for all rG-CSF-treated groups were greater than the mean CPA values at all sampling times during the study. Lenograstim caused greater increases in ANCs than did filgrastim, with the maximal increase (+18.4-fold) being observed in the

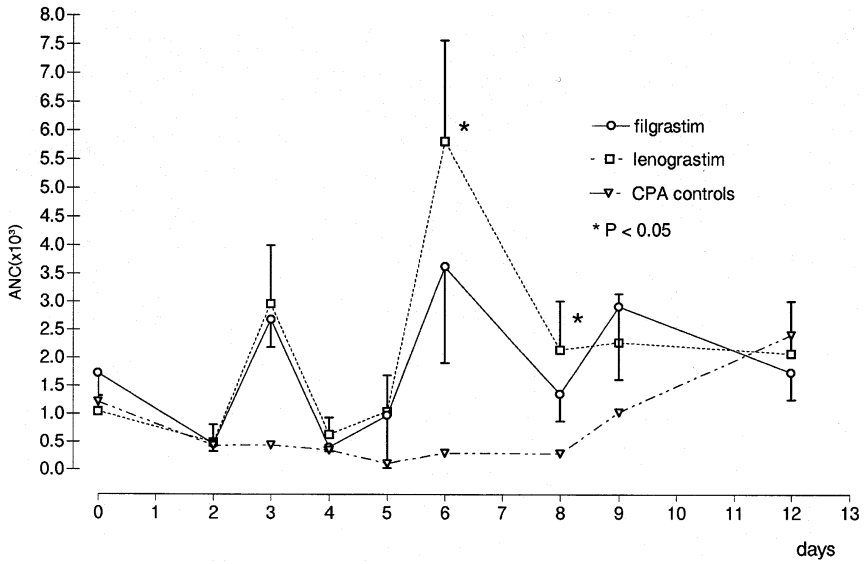

Fig. 5 Mean ANCs ($\times 10^3/\mu l$) obtained in male CD rats treated with 50 mg/kg CPA (day 0) followed by four daily subcutaneous doses (days 1–4) of 100 μg/kg filgrastim or lenograstim

100-μg/kg group on day 6 ($P < 0.05$ versus the filgrastim group). Statistically significant differences in ANCs between lenograstim- and filgrastim-treated groups were also noted on day 3 (30 μg/kg) and day 8 (100 μg/kg). The calculated $AUC_{day\ 1-12}$ (area under the curve of the mean ANC above the CPA-control baseline versus time) in the 30-μg/kg lenograstim group was 9.8 (10^3 cells/μl× days) as compared with a calculated AUC of 8.2 (10^3 cells/μl × days) for the group receiving 30 μg/kg filgrastim. The calculated $AUC_{day\ 1-12}$ for the 100-μg/kg lenograstim group was 16.75 (10^3 cells/μl × days) as compared with 12.45 (10^3 cells/μl × days) for the group receiving 100 μg/kg filgrastim.

Other WBC parameters

Normal rats. Dose-related increases in the absolute number of large unclassified leukocytes (LUCA) were observed on day 3 in groups treated with lenograstim at 30 or 100 μg/kg per day and on day 5 in groups treated daily with 30 or 100 mg/kg lenograstim or filgrastim. The maximal increase (+3.9-fold) the meaerved on day 5 in the 100-mg/kg lenograstim group; on this day the LUCA count noted for the 30-mg/kg lenograstim group (+2.8-fold) was comparable with the count recorded for the 100-mg/kg filgrastim group. Values returned to the reference range by day 8. Both compounds marginally increased the absolute number of eosinophils on days 2 and 3 of the study; these values returned to the reference range by day 5. Lymphocyte, monocyte, and basophil counts were unaffected by rHuG-CSF administration.

Neutropenic rats. Mean absolute lymphocyte counts obtained in all CPA-treated groups were moderately to markedly decreased relative to the mean saline control values on all sampling dates (maximum −85% on day 5). Although lymphocyte counts increased in all groups on day 12, they did not attain levels comparable with control values. The mean absolute LUCA counts obtained in all CPA-treated groups were decreased as compared with saline control values on days 2–5. Dose-related increases in mean LUCA counts were observed on day 6, with lenograstim-treated groups responding more strongly (maximum +2.7-fold in the 100-mg/kg lenograstim group). Variable non-dose-related changes in this parameter were observed throughout the remainder of the study. Mean eosinophil and basophil counts were decreased relative to saline control values but remained within the reference range throughout the study. Mean monocyte counts fell to below or near the lower limit of the reference range until Day 12, when they returned to the reference range.

When groups treated with filgrastim or lenograstim were compared with the group given CPA only, variable non-dose-related changes were observed in mean absolute lymphocyte counts throughout the study. Dose-related increases in mean absolute LUCA counts were observed on days 3, 5, and 6 in all rHuG-CSF-treated groups. The increases were comparable between the compounds at most sampling times except for day 6, when the LUCA count obtained in the 100-μg/kg lenograstim group (+5.2-fold) was more elevated than that recorded for the 100-μg/kg filgrastim group (+3.6-fold). Variable non-dose-related changes in this parameter were observed throughout the remainder of the study. Variations in mean eosinophil, basophil, and monocyte counts could not be attributed to rG-CSF administration.

RBC parameters

No compound-related effect was observed with filgrastim or lenograstim in normal (nonneutropenic) rats. In all groups pretreated with CPA, minimal to mild decreases in

mean RBC counts as compared with mean saline control values were recorded on days 5–12 of the study (maximum −23% on day 9), with no relation being found to the compound given (CPA only, filgrastim, or lenograstim).

With regard to platelet counts, no compound-related effect was observed following filgrastim or lenograstim treatment in normal (nonneutropenic) rats. In all groups pretreated with CPA, minimal to moderate decreases in mean platelet counts as compared with mean saline control values were recorded on day 2 and on days 4–9 of the study (maximum −62% on day 6), with no relation being found to the compound given (CPA only, filgrastim, or lenograstim) or to the dose of rG-CSF delivered. When groups receiving filgrastim or lenograstim were compared with the group given CPA only, minimal to mild decreases in mean platelet counts (non-dose-related) were recorded on days 2, 4, 6, 8, and 12 (maximum −24% on day 6). Mild to moderate increases were recorded on days 5 and 9 (maximum +48% on day 5). Although lenograstim was associated with greater increases in platelet counts than was filgrastim, the increases were not always dose-related.

Discussion

In contrast to the variability of the responses noted in human patients [11, 15], both normal and neutropenic CD rats responded homogeneously to treatment with rG-CSF, suggesting that this species is suitable for measurement of the in vivo potency of rG-CSF. At all dose levels tested, both forms of rG-CSF produced a clear elevation in ANC on days 2, 3, and 5 in normal animals and on days 4–9 in neutropenic animals. In normal animals the increase in ANC was progressive and dose-related, with the highest values occurring 24 h following the fourth administration of rG-CSF, i.e., on day 5. In neutropenic rats, treatment with rG-CSF appeared to shorten the period of significant (ANC $< 0.5 \times 10^3/\mu l$) neutropenia.

With the exception of a single mean value, mean ANC values obtained in nonneutropenic rats receiving lenograstim were consistently higher than the respective values recorded for filgrastim-treated groups; the difference in mean ANCs observed between filgrastim- and lenograstim-treated groups increased with progressive treatment, was marginal on day 2, became moderate and statistically significant on day 3 at 30 and 100 μg/kg, and was statistically significant on day 5 at all dose levels tested. On days 3 and 5 the mean ANC of the groups treated with 30 μg/kg lenograstim was comparable with the ANC observed following treatment with 100 μg/kg filgrastim, suggesting a biological equivalence of these doses. A comparison of the area under the ANC-time curve indicated that lenograstim had 30–40% greater potency in neutrophil mobilization as compared with filgrastim.

In neutropenic rats an initial increase in WBC and ANC counts was observed in animals treated with both forms of rG-CSF on day 3. This increase was somewhat more prominent in lenograstim-treated groups (statistically significant in the 30-μg/kg lenograstim group), and it may be attributed to the emergence of mature neutrophils from the storage pool of the bone marrow secondary to the CPA insult delivered on day 0 of the study [22, 23].

The subsequent decrease in WBC and ANC counts on days 4 and 5 was probably due to a combination of depletion of the marrow storage pool, depletion of WBC precursors in the maturation phase due to CPA treatment, and the normal rapid loss of neutrophils from the circulating pool of the peripheral blood. This decrease was followed by a steep increase in ANC values, culminating on day 6 when rG-CSF-treated groups had increased ANCs of up to +19-fold (100-μg/kg lenograstim group) as compared with the CPA-treated control group. The maximal response to both rG-CSF preparations occurred during study days 5–8. With the exception of a single value obtained during the principal response period (30 μg/kg lenograstim, day 6) and two minimally lower values recorded during the post-response period, lenograstim-treated groups had consistently higher ANC values than did the groups receiving filgrastim, the difference reaching statistical significance on day 3 in the groups treated at 30 μg/kg per day and on days 6 and 8 in the groups treated at 100 μg/kg per day. In addition, the calculated areas under the ANC curves were 20–35% higher for lenograstim than for filgrastim. These results demonstrate that the activity of lenograstim is greater than that of filgrastim in neutropenic animals.

Increased LUCA counts in normal and in neutropenic rats were observed along with increased ANCs in all rG-CSF groups. Although increases severalfold in magnitude appear striking, it must be stated that these cells generally comprised less than 3% of the pretreatment circulating WBC population in each study. The maximal rG-CSF-induced increase found in either study represented less than 8% of the total WBC population on a given sampling date. Moreover, as no visual examination of blood samples was performed, the cell type(s) contributing to increased LUCA counts could not be defined. We attribute this finding to a nonspecific stimulatory action of rG-CSF on the bone marrow. Again, the LUCA counts obtained in lenograstim-treated groups were higher than those recorded for filgrastim-treated groups, further supporting the superior activity of lenograstim in this study.

Our results confirm the greater potency of lenograstim as compared with filgrastim, which was recently reported in in vitro human bone marrow assays [10] and in a recent study in healthy human volunteers [20]. The difference in the in vivo activity of lenograstim may be due to the greater stability of the glycosylated rG-CSF against proteases or other degradation/denaturation processes [8, 9, 16]. In a previous study, rats treated with glycosylated rG-CSF had detectable plasma levels of GSF for up to 10 h after administration, whereas following similar doses of nonglycosylated rG-CSF, blood levels were scarcely detectable [13]. Both a higher and more extended blood concentration and a greater intrinsic in vivo potency of the glycosylated form of rG-CSF may result in enhanced hematopoietic stimulation.

In conclusion, in normal CD rats, four subcutaneous treatments of 10, 30, or 100 μg/kg lenograstim produced a dose-related and cumulative increase in neutrophil count that was consistently and significantly higher than the response observed after administration of filgrastim. In neutropenic CD rats, four subcutaneous doses of 30 or 100 μg/kg lenograstim produced consistently higher activity than did corresponding doses of filgrastim. These results confirm that in normal and neutropenic male CD rats, lenograstim, the glycolysated form of rG-CSF, has greater biological potency than does filgrastim, the nonglycosylated form of rG-CSF.

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