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Levels of Recombinant Human Granulocyte Colony-Stimulating Factor in Serum Are Inversely Correlated with Circulating Neutrophil Counts

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Recombinant human granulocyte colony-stimulating factor (rhG-CSF) is effective in countering chemotherapy-induced neutropenia. However, serum rhG-CSF levels cannot be maintained throughout the course of rhG-CSF therapy. The drop in serum rhG-CSF levels may vary with the duration of rhG-CSF administration or with the circulating neutrophil counts. We investigated the relationship between serum G-CSF levels and circulating neutrophil counts and the pharmacokinetics of rhG-CSF for patients with lung cancer who had been treated with myelosuppressive chemotherapy and then with subcutaneous rhG-CSF (lenograstim, 2 μg per kg of body weight per day). Twelve patients were randomly assigned to four groups with different rhG-CSF therapy schedules. Serum G-CSF levels were measured by an enzyme immunoassay method. Serum G-CSF levels during the rhG-CSF therapy greatly exceeded endogenous G-CSF levels and were mainly due to the presence of exogenous rhG-CSF rather than increased levels of endogenous G-CSF. Despite the duration of rhG-CSF administration, serum G-CSF levels during rhG-CSF therapy were inversely correlated with circulating neutrophil counts ($r^2 = 0.73$, $P < 0.0001$). The value for the area under the concentration-time curve of rhG-CSF on the day of neutrophilia was lower than that on the day of neutropenia ($P < 0.05$). Our results suggest that the fall in serum G-CSF levels during rhG-CSF therapy may result from increased clearance and/or decreased absorption of rhG-CSF, two processes related to circulating neutrophil counts.

Recombinant human granulocyte colony-stimulating factor (rhG-CSF) is effective in countering chemotherapy-induced neutropenia (1). Recent studies have demonstrated that serum rhG-CSF levels cannot be maintained at a steady level throughout rhG-CSF therapy but rather decrease during treatment (9, 12). This phenomenon is thought to be associated with receptor-mediated clearance by neutrophils, unknown mechanisms of clearance by other organs, or absorption of rhG-CSF from the injected site (9, 12, 22). However, the true mechanism which accounts for the change in serum rhG-CSF levels has not yet been determined. To clarify whether the drop in serum rhG-CSF levels varies with the length of rhG-CSF administration or with the number of circulating neutrophils, we examined serum G-CSF levels in patients with lung cancer receiving rhG-CSF by different protocols following chemotherapy.

MATERIALS AND METHODS

Patient selection. This study was conducted in accordance with institutional ethical standards. The following inclusion criteria were used for patient selection: (i) the presence of histologically or cytologically confirmed non-small-cell lung cancer according to the typing criteria of the World Health Organization (25); (ii) an age of below 75 years; (iii) an Eastern Cooperative Oncology Group performance status of 2 or better (27); (iv) the absence of both brain and bone marrow metastases; (v) adequate bone marrow function, with a neutrophil count of >2,000/μl, a platelet count of >100,000/μl, and a hemoglobin level of >10 g/dl; (vi) normal hepatic and renal functions; and (vii) the informed consent of the patient to participate in the study. Computed tomography and bone scintigraphy were performed to determine the patients' stages of cancer according to the criteria of the International Union against Cancer (15). Twelve consecutive patients were selected for analysis on the basis of the above criteria.

Treatment schedule. Patients received one cycle of MVP therapy: 8 mg of mitomycin per m^2 on day 1, 3 mg of vindesine per m^2 on days 1 and 8, and 80 mg of cisplatin per m^2 on day 1. rhG-CSF (2 μg per kg of body weight; lenograstim; Chugai Pharmaceutical Company, Tokyo, Japan) was administered subcutaneously at 9 a.m. on the scheduled days. Lenograstim is glycosylated rhG-CSF purified from Chinese hamster ovary cells (17), and the dose of 2 μg/kg was selected on the basis of results of a dose determination study with patients receiving MVP therapy (18).

Patients were randomly assigned to four groups, which differed in their rhG-CSF schedules. The control group consisted of three patients receiving no rhG-CSF treatment. Three patients in the early prophylaxis group received rhG-CSF on day 2 of the MVP cycle. rhG-CSF was administered on day 8 of the MVP cycle to the late prophylaxis group ($n = 3$), while the therapeutic group ($n = 3$) received rhG-CSF on the first day of neutropenia. Neutropenia was defined as a neutrophil count of <1,000/μl. The administration of rhG-CSF was discontinued when the neutrophil count exceeded 5,000/μl after the nadir.

Blood samples. In order to study the serial changes in circulating neutrophil counts and serum G-CSF levels, blood samples were obtained just before 9 a.m. every 3 days after the MVP therapy for control patients and rhG-CSF therapy groups. In addition, to study the pharmacokinetics of rhG-CSF, blood samples were obtained before and 3, 6, 12, and 24 h after rhG-CSF administration on the first day of neutropenia (<1,000/μl) and on the day of neutrophilia (>5,000/μl) of the three patients of the therapeutic group.

The number of neutrophils was counted by placing the blood samples into tubes containing EDTA. Total leukocyte counts were determined with an automated hematology analyzer (model NE8000; Toa-Iyo Denshi Company, Kobe, Japan), and differential cell counts were performed by examining 100 leukocytes on blood smears. Neutrophil counts were obtained by multiplying the total leukocyte count by the percentage of neutrophils.

Serum was obtained by centrifugation immediately after blood collection, and all serum samples were stored at −20°C until assayed. Serum G-CSF levels were measured by an enzyme immunoassay (14, 24). The reproducibility of the enzyme immunoassay results was confirmed with intra- and interassay coefficients; the variance ranged from 4.3 to 6.2% and 6.2 to 9.0%, respectively (14). Serum G-CSF levels during the rhG-CSF therapy include endogenous G-CSF and rhG-CSF levels, while the levels measured prior to rhG-CSF therapy reflect those of endogenous G-CSF only.

Statistical analysis. In the study of serial changes in circulating neutrophil counts and serum G-CSF levels, the values were log transformed for normaliza-

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TABLE 1. Patient characteristics

Treatment group[a]	Age range	No. of patients of indicated sex		No. of patients with performance status:		No. of patients with indicated histology[b]	
		Male	Female	0	1	Adeno	Squamous
Control	52–62	3	0	1	2	1	2
Early prophylaxis	66–67	3	0	0	3	1	2
Late prophylaxis	48–74	2	1	1	2	3	0
Therapeutic	60–76	3	0	0	3	1	2

[a] Patients in the early and late prophylaxis groups and in the therapeutic group received rhG-CSF on days 2 and 8 of MVP therapy and on the first day of neutropenia. The number of patients in each group was three.

[b] Adeno, adenocarcinoma; squamous, squamous cell carcinoma.

tion. The correlation between neutrophil counts and G-CSF levels was examined by linear regression analysis. The coefficient of determination (r^2) was used to assess the variability in G-CSF levels explained by neutrophil counts (8). In the study of the pharmacokinetics of rhG-CSF, the area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) was calculated by trapezoidal rule. Following the log transformation of AUC_{0-24} values, the difference in AUC_{0-24} values for the two days was statistically tested by Student's paired t test. A two-tailed P of <0.05 was considered significant.

RESULTS

The characteristics of patients in each group are summarized in Table 1. All patients had stage IV disease and had not received prior chemotherapy. There were no marked differences in clinical characteristics among different groups, except for a higher proportion of adenocarcinomas in the late prophylaxis group. No patient had renal or hepatic dysfunction, fever, or infection during the study period.

As shown in Fig. 1A, serum G-CSF (endogenous G-CSF) levels increased when neutrophil counts decreased, and they reached approximately 10^2 pg/ml. Serum G-CSF (endogenous G-CSF plus rhG-CSF) levels rose rapidly after the initiation of rhG-CSF administration (Fig. 1B to D), and the peak levels greatly exceeded those of endogenous G-CSF observed in Fig. 1A. Although the G-CSF levels peaked at more than 10^3 pg/ml on the day of the nadir, the levels decreased during the neutrophil recovery phase. The periods from the initiation of rhG-CSF therapy to the fall in G-CSF levels were 12 days for the early prophylaxis group, 6 to 8 days for the late prophylaxis group, and 4 to 5 days for the therapeutic group. G-CSF levels decreased during the neutrophil recovery phase irrespective of the duration of rhG-CSF administration.

There was a significant negative correlation between serum G-CSF (endogenous G-CSF plus rhG-CSF) levels during the rhG-CSF therapy and the circulating neutrophil counts of each therapy group ($P < 0.0001$), as shown in Fig. 2A to C. The regression lines for different therapy groups were quite similar; the duration of rhG-CSF administration did not influence the negative correlation between serum G-CSF levels during rhG-CSF therapy and circulating neutrophil counts. When data from the three groups receiving therapy were plotted together (Fig. 2D), the serum G-CSF levels during rhG-CSF therapy were inversely correlated with circulating neutrophil counts ($P < 0.0001$), and the latter accounted for 73% of the variability observed in serum G-CSF levels.

The concentration-time curves of rhG-CSF are shown in Fig. 3. The AUC_{0-24} on the day of neutrophilia (mean, 8,912 pg ·

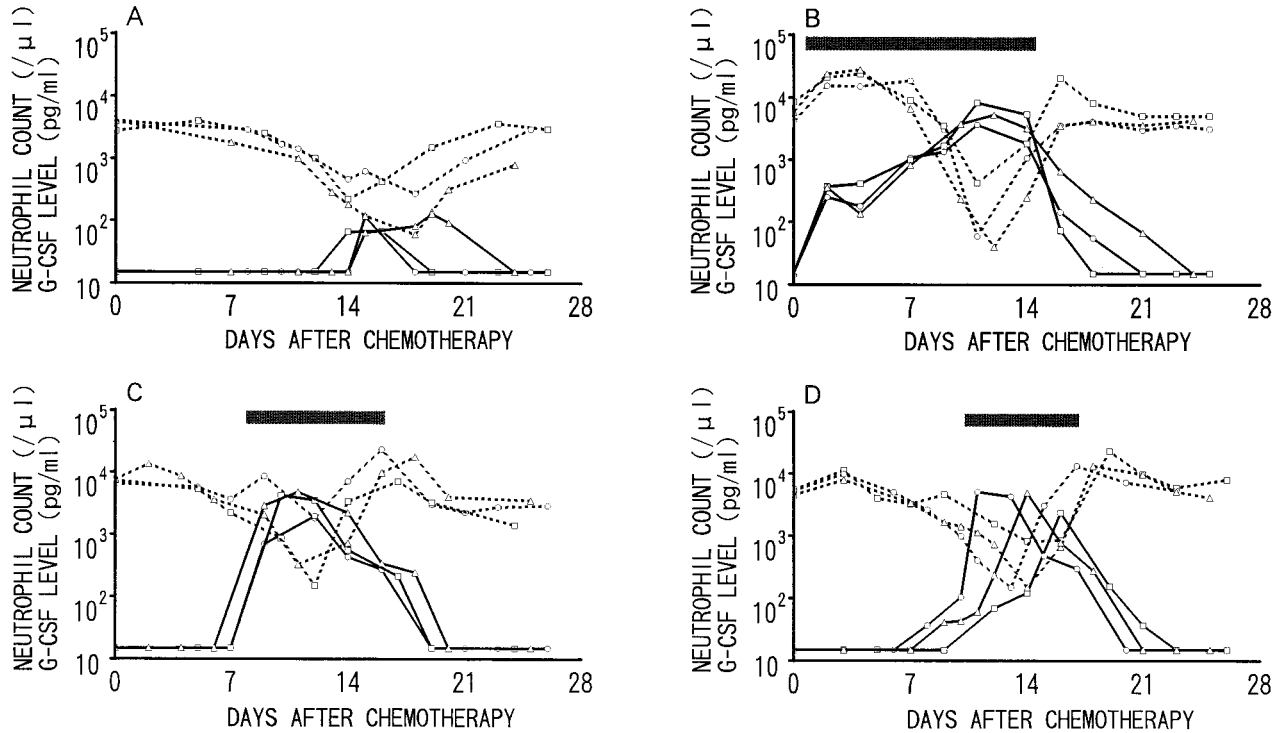

FIG. 1. Serial changes in serum G-CSF levels (solid lines) and blood neutrophil levels (broken lines) for each patient according to the following schedules: the control group (A), the early prophylaxis group (B), the late prophylaxis group (C), and the therapeutic group (D). G-CSF and neutrophil levels are plotted on the same log scale. The same symbols indicate data from the same patient. Thick horizontal bars indicate periods of rhG-CSF administration to the patients whose symbol is a circle.

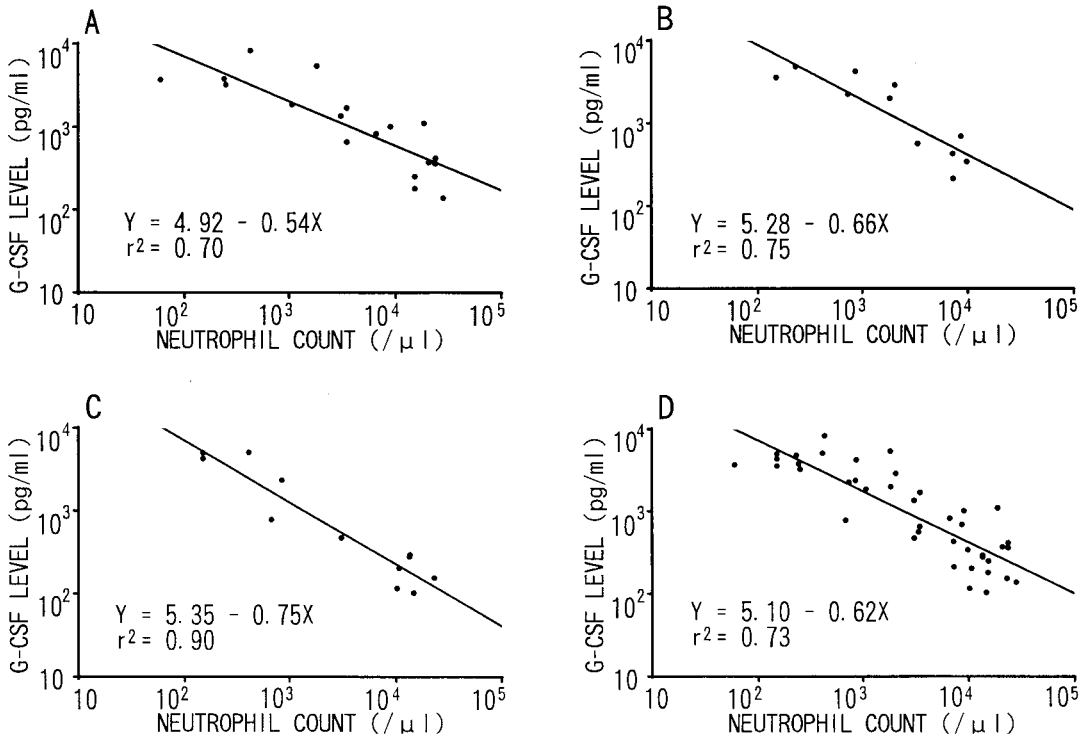

FIG. 2. Relationship between serum G-CSF levels and neutrophil counts during rhG-CSF administration. (A) Early prophylaxis group. (B) Late prophylaxis group. (C) Therapeutic group. (D) The three therapy groups plotted together.

h/ml) was significantly lower than that on the day of neutropenia (mean, 80,217 pg · h/ml) ($P < 0.05$).

DISCUSSION

This study demonstrated that the drop in serum G-CSF levels during rhG-CSF therapy is closely associated with the circulating neutrophil count, irrespective of the duration of rhG-CSF administration. Furthermore, our results also demonstrated that the AUC_{0-24} values of rhG-CSF decreased on the day of neutrophilia compared with those on the day of neutropenia. These findings suggest that the fall in serum G-CSF levels is due to increased clearance and/or decreased absorption, two processes that are related to the circulating neutrophil count.

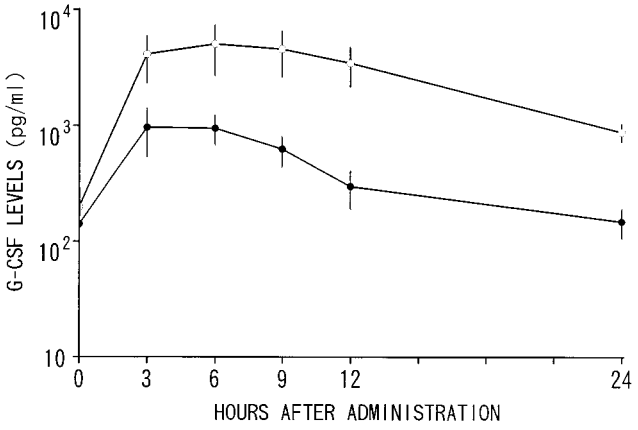

FIG. 3. Pharmacokinetics of rhG-CSF administered on two days when patients experienced different neutrophil counts. Open and closed circles show the kinetics on the day of neutropenia (mean ± standard deviation for neutrophil counts, 556 ± 193/μl) and neutrophilia (11,853 ± 1,993/μl), respectively. Each point with the vertical bar represents a mean ± the standard deviation.

In consideration of serum rhG-CSF levels, the contribution of endogenous G-CSF is an important issue. Endogenous G-CSF can be detected in patients with neutropenia, fever, infection, and renal or hepatic dysfunction (4, 19) but is usually not detected by enzyme immunoassay when the G-CSF level is <30 pg/ml (24). Furthermore, rhG-CSF itself may activate other endogenous cytokines which further stimulate the production of endogenous G-CSF. However, in this study, none of the patients had renal or hepatic dysfunction, fever, or infection. In addition, several preclinical studies, using radiolabeled and nonlabeled rhG-CSF, demonstrated that serum G-CSF levels after the administration of nonlabeled rhG-CSF were somewhat similar to the levels of radioactivity in serum following the administration of labeled rhG-CSF (10, 11). Thus, it is unlikely that exogenously administered rhG-CSF directly or indirectly stimulates endogenous G-CSF. Since serum G-CSF levels during rhG-CSF therapy greatly exceeded endogenous G-CSF levels (Fig. 1B to D), levels in serum reflected mainly exogenous rhG-CSF. The change in serum G-CSF levels during rhG-CSF therapy did not result from a change in endogenous G-CSF levels induced by neutropenia.

Undefined mechanisms of the elimination of rhG-CSF and unknown local kinetics of rhG-CSF in the subcutaneous tissue complicate the interpretation of serum rhG-CSF levels. As for other drugs, the kidney is considered the major elimination site of rhG-CSF (23). However, G-CSF is known to be eliminated through the receptors on neutrophilic lineage cells (16), and mature neutrophils have the greatest number of G-CSF receptors among neutrophilic lineage cells (7). Layton et al. (12) reported that the elimination half-time of intravenously admin-

istered rhG-CSF is prolonged at a dose of 10 μg/kg or more, suggesting a saturable mechanism of clearance. In addition, the clearance mechanism appears to be saturated on the day of neutropenia (12). The saturable mechanism of clearance is thought to be associated with a receptor-mediated clearance by neutrophils.

Similar clearance mechanisms have been observed with studies of rh macrophage-CSF, rhG-macrophage-CSF, and rh erythropoietin (rhEPO) (2, 3, 5, 13, 20). The clearance rates of rh macrophage-CSF and rhG-macrophage-CSF vary with the number of monocytes and neutrophils, respectively (2, 3, 5, 20). In addition, during rhEPO therapy, serum EPO levels remain stable in responders but increase in nonresponders (13), suggesting that the stimulated erythropoiesis in responders consumes rhEPO.

The local kinetics of rhG-CSF in the subcutaneous tissue, where rhG-CSF is usually injected, is an additional problem. The bioavailability of rhG-CSF after subcutaneous administration is considered to be approximately 30% (6). However, myelosuppressive chemotherapy or rhG-CSF administration changes the number of tissue neutrophils and the amount of blood in the subcutaneous compartment (26) and the levels of absorption of rhG-CSF may differ between the days of neutropenia and neutrophilia. Stute et al. (22) reported that the apparent clearance rate after subcutaneous administration increases at lower doses of rhG-CSF, which is possibly related to differences in absorption rates and not elimination rates. Thus, since the level of absorption may vary and depends on a variety of factors, the pharmacokinetics of intravenous and subcutaneous administration should be thoroughly investigated to resolve the above-described problem.

What are the clinical implications of our study? It is clear that the effect of rhG-CSF cannot always be evaluated by the achieved serum G-CSF levels. In our previous study, the late prophylaxis schedule of rhG-CSF administration was more effective in countering neutropenia following MVP therapy than the early prophylaxis or therapeutic schedules (21). In this study, no characteristic change in serum G-CSF levels was observed for patients in the late prophylaxis group compared with those of the other groups (Fig. 1B to D). Further studies are necessary to clarify the relationship between rhG-CSF and granulopoiesis.

Patients recruited for our study were selected consecutively without any selection bias, but their number was small. However, the differences were statistically too marked to have resulted from chance. Accordingly, we think that the small number of patients does not significantly influence the outcome of the study.

In conclusion, serum rhG-CSF levels are inversely correlated with the circulating neutrophil counts. Our results indicate that circulating neutrophil counts may be related to systemic clearance and/or absorption of subcutaneously administered rhG-CSF.

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