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Subcutaneous Administration of Recombinant Human Granulocyte Colony-stimulating Factor (KRN8601) in Intensive Chemotherapy for Patients with Advanced Lung Cancer

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The efficacy and toxicity of recombinant human granulocyte colony-stimulating factor (rh G-CSF, KRN8601) given subcutaneously was evaluated in patients with advanced lung cancer undergoing intensive chemotherapy. Twenty-nine and 30 patients with or without prior therapy were enrolled in this study. At dose levels of 50, 90 and 130 $\mu g/m^2$ of rh G-CSF for 14 consecutive days after chemotherapy, the mean neutrophil nadir counts, the mean neutrophil nadir ratios and the duration of neutropenia (days of $<1000/mm^3$) were significantly improved. No significant differences were seen in frequency and duration of febrile episodes ($>38°C$). When rh G-CSF is given subcutaneously, the dose required for an equal effect in alleviating neutropenia is 50% of that required when it is given intravenously. The monocyte counts in the peripheral blood were also significantly increased after chemotherapy cycles with rh G-CSF. The cumulative plasma concentration of rh G-CSF showed a decrement after 7–9 days despite maintenance of the same dose of rh G-CSF for the entire 14 days. In conclusion, 50–130 $\mu g/m^2$ of sc rh G-CSF increased the neutrophil nadir count and shortened the duration of neutropenia in patients undergoing intensive chemotherapy for lung cancer without intolerable side effects.

Key words: Recombinant human granulocyte colony-stimulating factor — Chemotherapy — Lung cancer

The number of deaths from lung cancer is over 30,000 annually in Japan and has increasing year by year.[1)] Many cases of lung cancer are found in advanced stages despite efforts for early detection. Innovative clinical trials of combined modalities, including intensive chemotherapy, are mandatory if we are to improve treatment results in lung cancer.

Myelosuppression is the most important dose-limiting toxicity of many chemotherapy regimens. Recently, recombinant human colony-stimulating factors (rh CSFs) have been employed in patients receiving intensive chemotherapy with or without bone marrow transplantation.[2-5)] Although the usefulness of rh CSFs in reducing the risk of fatal complications has been demonstrated, the optimal dose and administration schedule of rh CSFs are still under study.

In a previous study,[6)] we reported the effect of rh G-CSF administered intravenously (iv) for chemotherapy-induced myelotoxicity in patients with advanced lung tumors. In the present study, we evaluated efficacy and toxicity of subcutaneously (sc) administered rh G-CSF in patients receiving intensive chemotherapy for lung cancer. We also studied the pharmacokinetics of sc rh G-CSF in serum using a radioimmunoassay (RIA) method (Takanashi *et al.*[6)]). Successive subcutaneous administrations of rh G-CSF at doses of 50–90 $\mu g/m^2$ were demonstrated to be useful for increasing the neutrophil nadir count and reducing the duration of neutropenia in patients with chemotherapy-induced myelosuppression. When rh G-CSF was given subcutaneously, the dose required for a given neutropenia-alleviating effect was only half that in the case of iv rh G-CSF.

PATIENTS AND METHODS

Patient selection From August 1988 through September 1989, 59 patients with advanced lung cancer entered this study. Patients without histological or cytological confirmation, age >75 years old, PS(ECOG)4, with abnormal liver or renal function, with respiratory or heart failure, with a life expectancy of less than two months, or without informed consent were excluded. Patients with normal hematological function (WBC $>4\times10^3/mm^3$, Hb >10 g/dl, platelets $>10\times10^3/mm^3$) were entered into this study. The ethics committee of the National Cancer Center Hospital gave its approval for this protocol study to be conducted.

rh G-CSF rh G-CSF (KNR8601) was kindly provided by Kirin and Sankyo Co. (Tokyo).[6)]

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Clinical monitoring Complete blood cell counts and differential counts were performed three times per week during neutropenia. Urinalysis, liver and renal function tests were carried out twice a week. Chest X-rays were taken once a week to evaluate the response to chemotherapy. In 12 patients the pharmacokinetics of rh G-CSF were evaluated by a double antibody RIA method.[6] To evaluate the cumulative plasma concentration of rh G-CSF, blood samples were obtained in the early morning before that day's administration of rh G-CSF every two days for 15 consecutive days. The formation of neutralizing antibodies to rh G-CSF was studied in blood samples taken before and two weeks after administration of rh G-CSF, by the RIA method.

Study design Two days after chemotherapy, patients were given rh G-CSF subcutaneously for 14 consecutive days. The starting dose of rh G-CSF was 18 $\mu g/m^2$ and after initial evaluation of toxicity the doses were escalated to 50, 90 and 130 $\mu g/m^2$. At least five patients were accrued at each dose level. In patients with prior therapy the doses of rh G-CSF were 50, 90, 130 and 200 $\mu g/m^2$. If any WHO grade III–IV toxicity was observed, three patients at the same dose were additionally scheduled in order to evaluate the extent of the side effects. None of the individual patients received an escalated dose.

The chemotherapy regimens used were as follows: for non-small cell lung cancer; vindesine (VDS) (3 mg/m^2) +cisplatin (CDDP) (80 mg/m^2) or VDS+CDDP+ mitomycin (MMC) (8 mg/m^2); for small cell lung cancer, CDDP + etoposide (VP-16) (300 mg/m^2), cyclophosphamide (CPA) (1 g/m^2) + adriamycin (ADM) (40 mg/m^2)+vincristine (VCR) (1 mg/m^2), alternating courses of these two regimens or CDDP+ ADM+CPA+VP-16 (PACE). In order to compare the effect of sc rh G-CSF with or without chemotherapy, patients without prior therapy were scheduled to receive a first cycle of chemotherapy without rh G-CSF and the second cycle of the same regimen with rh G-CSF. If patients achieved a partial response, they were given two or three more cycles of the same chemotherapy. Patients with prior chemotherapy were scheduled to receive a first cycle of chemotherapy with rh G-CSF and the second cycle of the same regimen without rh G-CSF. Steroids or other immunomodulating drugs were prohibited during the study.

Statistical analysis The pharmacokinetic data for rh G-CSF were analyzed using the program NONLIN 84. Student's t test was carried out to determine if there were significant differences in the absolute neutrophil nadir count, the neutrophil nadir ratio and the duration of neutropenia during chemotherapy cycles with or without rh G-CSF at each dose level.

RESULTS

Characteristics of patients The characteristics of the patients enrolled in this study are shown in Table I. Thirty patients had received no prior therapy and all of them had non-small cell lung cancer. Twenty-nine patients had had prior therapy. Three patients had received chemotherapy+surgery, two patients had had surgery+ radiation, four patients had received radiation+chemotherapy and the other 20 had been given chemotherapy alone before accrual to this study. Eleven of the 29 (38%) previously treated patients had small cell lung cancer.

Comparison of chemotherapy-induced neutropenia with or without rh G-CSF The results for patients without prior therapy who received the same chemotherapy regimen with or without rh G-CSF are shown in Table II. The neutrophil nadir ratios were calculated by dividing the neutrophil nadir count by the neutrophil count prior to chemotherapy administration. There were significant differences in the mean absolute neutrophil nadir counts, the mean neutrophil nadir ratios and the mean durations of neutropenia (days of $< 1000/mm^3$) between the chemotherapy cycles with rh G-CSF and those without rh G-CSF at dose levels of 50, 90 and 130 $\mu g/m^2$. Febrile episodes (38°C<) were observed in 7 (23%) and 9 (30%) of 30 patients with no prior therapy during chemotherapy cycles with or without rh G-CSF, respectively. Total numbers of days with febrile episodes were 13 and 20 with or without rh G-CSF, respectively (not significant). Between dose levels (18 vs. 50, 90, 130, 50 vs. 90, 130, 90 vs. 130 $\mu g/m^2$), there were no significant differences in the mean nadir counts in chemotherapy with rh G-CSF (Table II). In the mean neutrophil nadir ratio in chemotherapy with rh G-CSF, there was a significant difference between the dose levels of 18 and 90 $\mu g/m^2$ ($P<0.05$, two-sided).

Effects of rh G-CSF on white blood cell differentials Table III illustrates the effect of rh G-CSF on the mean maximum counts of neutrophils, monocytes and lymphocytes in the peripheral blood of previously untreated patients during courses of chemotherapy. For the mean maximum neutrophil count, there were significant differences between dose levels of 18 vs. 50, 18 vs. 90 and 18 vs. 130 $\mu g/m^2$ ($P<0.05$, two-sided). No significant differences was seen in the mean maximum neutrophil count between dose levels of 50 vs. 90, 50 vs. 130 or 90 vs. 130 $\mu g/m^2$. Compared with the results of chemotherapy without rh G-CSF, there were significant increases in the mean maximum monocyte counts and the mean maximum neutrophil counts at all dose levels of rh G-CSF. At dose levels of 90 and 130 $\mu g/m^2$, there were significant increases in the mean maximum lymphocyte counts in

Table I. Characteristics of Patients

		Without prior Tx	With prior Tx
No. of patients		30	29
Age	Mean (range)	56.9 (36–74)	64.0 (45–75)
Sex	Male	26	21
	Female	4	8
Performance status (ECOG)			
	0–1	27	21
	2–3	3	8
Metastasis	M0	9	10
	M1	21	19
Cx regimen	MMC+VDS+CDDP	16	13
	CDDP+VDS	14	2
	PACE		8
	PVP		5
	CAV. PVP		1
Histology	AD	23	13
	SQ	4	3
	AD-SQ	1	
	LA	2	2
	SMALL		11

Abbreviations: Tx, treatment; Cx, chemotherapy; MMC, mitomycin; VDS, vindesine; CDDP, cisplatin; PACE, cisplatin+adriamycin+cyclophosphamide+VP16; PVP, cisplatin+VP16; CAV, cyclophosphamide+adriamycin+vincritine; AD, adenocarcinoma; SQ, squamous cell carcinoma; AD-SQ, adenosquamous cell carcinoma; LA, large cell carcinoma; SMALL, small cell carcinoma.

Table II. Responses to rh G-CSF in Intensive Chemotherapy for Lung Cancer in Patients without Prior Therapy

Dose of rh G-CSF ($\mu g/m^2$)	rh G-CSF	18	50	90	130
No. of patients		6	11	5	8
Absolute neutrophil nadir	+	1768±1598	2691±3447	2281±837	3173±4084
($/mm^3$)	−	961±711	734±804**	654±750*	345±260
Neutrophil nadir ratio	+	0.30±0.27	0.68±1.02	0.81±0.40	0.78±1.09
(NNR)	−	0.20±0.15	0.15±0.18	0.13±0.13**	0.06±0.05
Duration of neutropenia	+	1.8±3.6	0.6±1.6	0	1.5±2.8
($<1000/mm^3$) (days)	−	2.3±2.3	7.7±4.9*	6.4±4.4*	7.9±3.8*

NNR=neutrophil nadir/pretreatment neutrophil count.
Values are means±SD.
* $P<0.01$ two-sided. ** $P<0.05$ two-sided.

peripheral blood between chemotherapy cycles with or without rh G-CSF. But the mean maximum lymphocyte counts of chemotherapy cycles without rh G-CSF at dose levels of 90 and 130 $\mu g/m^2$ were lower than those of other dose levels, so it could not be concluded that rh G-CSF was active in increasing the maximum lymphocyte counts from these data.

Effect of rh G-CSF in patients with prior therapy The response to rh G-CSF in previously treated patients receiving intensive chemotherapy is shown in Table IV. Although the chemotherapy regimens and the characteristics of patients were heterogeneous (Table I), there was no apparent dose-response effect of rh G-CSF. At dose levels of 50, 90 or 130 $\mu g/m^2$ of rh G-CSF, the neutrophil

Table III. Dose-Response Relationship of rh G-CSF on WBC Differential in Intensive Chemotherapy for Lung Cancer

Dose of rh G-CSF ($\mu g/m^2$)	rh G-CSF	18	50	90	130
No. of patients		6	11	5	8
Maximum neutrophil count ($/mm^3$)	+	10436±4258	25323±17059	27141±8141	29746±15960
	−	4274±2181*	3546±2097*	2572±2633*	2240±1711*
Maximum monocyte count ($/mm^3$)	+	1242±495	2000±1174	2225±721	2115±1234
	−	662±165**	707±370*	678±353**	741±239**
Maximum lymphocyte count ($/mm^3$)	+	2291±1132	2950±1646	2192±1061	3082±2130
	−	2174±1456	1908±513	1312±552**	1542±1085**

Values are means±SD.
* $P<0.01$ two-sided. ** $P<0.05$ two-sided.

Table IV. Dose-Response Relationship of rh G-CSF in Intensive Chemotherapy for Lung Cancer in Patients with Prior Therapy

Dose of rh G-CSF ($\mu g/m^2$)	50	90	100	130	200
No. of total Cx courses	6	5	7	9	8
Absolute neutrophill nadir ($/mm^3$)	380±642	520±741	517±844	1124±2367	365±585
Maximum neutrophil count ($/mm^3$)	21267±12477	18622±16289	20643±5386	29000±19297	18983±12063
Duration of neutropenia ($<1000/mm^3$)(days)	3.0±2.6	3.6±2.1	4.6±2.3	3.5±1.6	3.4±2.2
Neutrophil nadir ratio (NNR)	0.127±0.243	0.178±0.291	0.137±0.206	0.215±0.384	0.078±0.093

Values are means±SD.

nadir ratios of patients with prior therapy were lower than those of patients without prior therapy. Although there were enough patients with prior therapy for comparison only at a dose level of 50 $\mu g/m^2$ of rh G-CSF, no obvious differences were seen in the mean absolute neutrophil counts, the mean neutrophil nadir ratios or the duration of neutropenia during chemotherapy cycles with or without rh G-CSF (data not shown).

Comparison of the pharmacokinetics and effects of rh G-CSF between iv and sc administration The pharmacokinetic parameters of plasma rh G-CSF were as follows: the mean AUC values (±SE) were 17.2±3.3, 51.6±9.0 and 112±11 μg h/liter at dose levels of 50, 90 and 200 $\mu g/m^2$, respectively. The mean $T_{1/2}$ values (±SE) were 5.0±0.7, 3.4±0.5 and 4.1±0.2 h at dose levels of 50, 90 and 200 $\mu g/m^2$, respectively. Fig. 1 shows the plasma concentraion curves of rh G-CSF after sc administration. The mean peak plasma concentrations in sc administration were achieved 4 to 6 h after injection of 50, 90 or 200 $\mu g/m^2$ of rh G-CSF. These concentrations were significantly lower than those achieved by iv administration.[6]

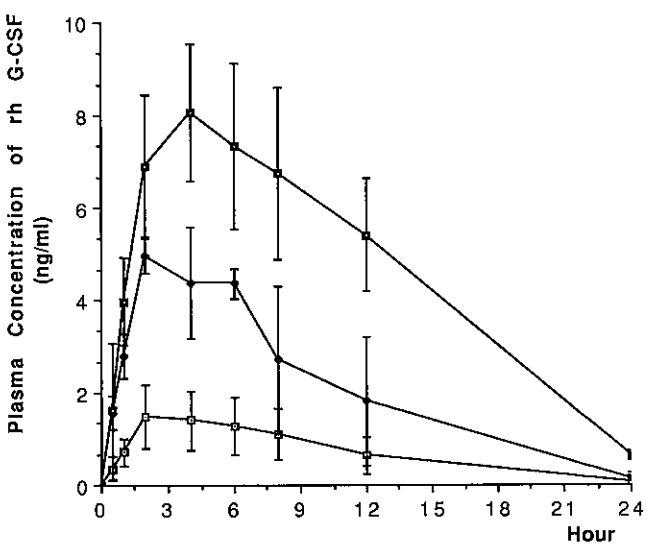

Fig. 1. Pharmacokinetic curve of plasma rh G-CSF after sc administration (mean±SD). (▣) 50 $\mu g/m^2$, (◆) 90 $\mu g/m^2$, (■) 200 $\mu g/m^2$.

Table V. Comparison of Maximum Neutrophil Count after Chemotherapy with rh G-CSF by sc and iv[a] Routes

Subcutaneous route					
Dose of rh G-CSF ($\mu g/m^2$)	18	50	90	130	
No. of patients	6	11	5	8	
Maximum neutrophil count	10436±4258	25323±17059	27141±8141	29746±15960	
Intravenous route[a]					
Dose of rh G-CSF ($\mu g/m^2$)	50	100	200	400	800
No. of patients	1	3	4	4	3
Maximum neutrophil counts	4484	6358±2328	38250±7852	43076±10519	47457±1089

All patients had had no prior therapy.
Values are means±SD.
a) Data from Eguchi *et al.*[6]

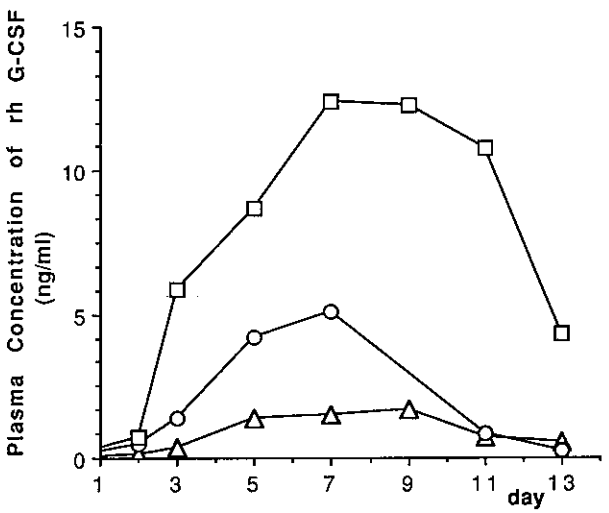

Fig. 2. The mean cumulative plasma concentration of rhG-CSF during sc administration for 14 consecutive days. Blood samples were obtained in the early morning before that day's administration of rh G-CSF every two days until day 15. (△) 50 $\mu g/m^2$, (○) 90 $\mu g/m^2$, (□) 200 $\mu g/m^2$.

The mean maximum neutrophil counts at each dose level after sc administration are compared with our previous results after iv administration[6] in Table V. Although this was not a randomized comparison, 50 $\mu g/m^2$ of rh G-CSF by sc administration produced effects similar to greater than 100 $\mu g/m^2$ by iv administration.

Cumulative plasma concentration of rh G-CSF during sc administration for 14 consecutive days Fig. 2 shows the mean cumulative plasma concentration of rh G-CSF. The plasma rh G-CSF levels increased until day 7–9. After that they decreased gradually although the administration of the same dose of rh G-CSF was continued until day 14. There was a negative correlation between the mean neutrophil counts in the peripheral blood and changes in the mean plasma concentrations of rh G-CSF (Pearson's correlation coefficient; $r=-0.99$ and $r=-0.64$ for the 3rd–7th and the 7–15th days, respectively, and when values were calculated with two-day lagging, $r=-0.96$ and $r=-0.82$ for each period).

Adverse effects associated with rh G-CSF administration No evidence of antibody formation against rh G-CSF was obtained by the RIA method until two weeks after discontinuation of sc rh G-CSF administration. Two patients had mild chest discomfort 3–6 h after the administration of rh G-CSF, but it subsided spontaneously and no abnormal changes were seen on the electrocardiogram. Mild elevation of serum lactate dehydrogenase (2N<) was transiently experienced by 39% (23/59) of the patients during the elevation of the peripheral neutrophil count and 0.5% (3/59) of the patient experienced mild elevation of serum alkaline phosphatase (2N<). All of these laboratory values returned to normal after the administration of rh G-CSF was finished, concurrently with the return of the peripheral blood cell count to baseline. These were no intolerable side effects of rh G-CSF administration in this study.

DISCUSSION

According to previous reports, rh G-CSF combined with intensive chemotherapy is effective for increasing the peripheral neutrophil nadir count and for shortening the duration of neutropenia. However, the optimal schedule and route of administration of rh G-CSF have not been determined. This study confirmed the effectiveness previously seen with rh G-CSF. Using an sc administration schedule, the effect of rh G-CSF was apparent, in a dose-related fashion, throughout the dose range of 18 to 130 $\mu g/m^2$.

As shown in Table III, rh G-CSF increased not only the neutrophil counts but also monocyte counts during

chemotherapy cycles with rh G-CSF. This suggests that rh G-CSF either can non-specifically induce monocyte proliferation or can act, in part, on a precursor to two lineages. *In vitro* studies of rh G-CSF demonstrated that it promotes differentiation of a monoblastic cell line synergistically with rh GM-CSF.[7] In previous clinical reports, moderate to high doses of rh G-CSF (10–60 μg/kg) by an sc or iv route induced monocytosis after intensive chemotherapy for cancer,[8–11] but could not shorten the duration of monocytopenia.[12]

Compared with patients without prior therapy, the absolute nadir counts and the neutrophil nadir ratios were lower in patients with prior therapy (Tables II and IV). Although the chemotherapy regimens were different in some patients, there was a trend of longer duration of neutropenia in patients with prior therapy, even if they received rh G-CSF, compared to the duration of neutropenia in the chemotherapy cycles with rh G-CSF in patients without prior therapy. The optimal dose of rh G-CSF to improve these parameters in previously treated patients could not be evaluated in this study. Morstyn *et al.* found no difference in duration of melphalan-induced neutropenia between patients with or without prior treatment, using 10 μg/kg of rh G-CSF given by continuous sc infusion.[11]

Similar effects on the degree and duration of neutropenia could be obtained at half the dose of rh G-CSF when using sc administration compared to iv administration. These results reflect differences in the pharmacokinetics of sc or iv administration of rh G-CSF. A cumulative effect of rh G-CSF was observed during 9 days of successive administration. However, the plasma concentration of rh G-CSF decreased rapidly 11 days after the beginning of rh G-CSF despite continued administration of rh G-CSF until day 14. This was a similar result to that reported by Layton *et al.*[11, 13] In patients with idiopathic aplastic anemia an inverse correlation between serum endogenous G-CSF level and blood neutrophil count was demonstrated by Watari *et al.*[14] In our study the onset of neutrophilia was between 10 and 12 days after starting sc administration of rh G-CSF. Whether a receptor for rh G-CSF is down-regulated or whether the proliferation of neutrophils absorbs increasing amounts of rh G-CSF is not clear from these data, but it would appear that continuing administration beyond 7–9 days is not useful.

In conclusion, it is clear that subcutaneous doses of rh G-CSF ranging from 50–130 μg/m^2 are effective in ameliorating the myelosuppression associated with moderately intensive chemotherapy. It may now be possible to explore the value of significant dose escalations in the generally older, sicker population of patients with lung cancer who can not tolerate autologous marrow rescue.

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