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Effect of recombinant granulocyte colony-stimulating factor (rG-CSF) on chemotherapy-induced neutropenia in patients with urogenital cancer

Toshihiko Kotake[1], Tsuneharu Miki[1], Hideyuki Akaza[2], Yoshinobu Kubota[3], Yasunori Nishio[4], Yosuke Matsumura[5], Kazuo Ota[6], and Nobuya Ogawa[7]

[1] Department of Urology, Center for Adult Diseases, Osaka; [2] University of Tokyo; [3] Yokohama City University; [4] Kyoto University; [5] Okayama University; [6] Nagoya Memorial Hospital; [7] Department of Pharmacology, Ehime University School of Medicine

Summary. The effects of recombinant granulocyte colony-stimulating factor (rG-CSF) on the myelosuppression, especially neutropenia, induced by cancer chemotherapy in patients with urogenital cancer were investigated in a randomized, controlled clinical study. In this study, rG-CSF was given subcutaneously at a dose of 2 μg/kg per day for 14 consecutive days. Changes in neutrophil counts were compared between the first (no rG-CSF) and second cycles (rG-CSF treatment period) of chemotherapy. rG-CSF administration was found to be effective in reducing the duration of neutropenia, in elevating the neutrophil nadir, and in reducing recovery time. Based on comparisons between the randomized rG-CSF treatment group (with rG-CSF) and the control group, treatment with rG-CSF resulted in the moderation or prevention of neutropenia and the acceleration of recovery. These results demonstrate that in chemotherapy of patients with urogenital cancer, in which neutropenia is a dose- or schedule-limiting factor, the concomitant use of rG-CSF may enable an increase in the dose (higher single dose or increased dose per unit of time) or shorten the chemotherapy period.

Table 1. List of institutions involved in the present study

Center for Adult Diseases, Osaka	Ehime University
University of Tokyo	Yokohama City University
Okayama University	Kyoto University
Asahikawa Medical College	Hokkaido University
Tohoku University	Sapporo Medical College
Nihon University	Yamagata University
Tokyo Women's Medical College	Chiba Cancer Center Hospital
National Cancer Center	Jikei University School of Medicine
Keio University	Cancer Institute Hospital
Kanagawa Prefectural Cancer Center	Saitama Cancer Center
University of Tsukuba	Niigata Cancer Center Hospital
Yamanashi Medical College	Nagoya Daini Red Cross Hospital
Hamamatsu University School of Medicine	Kanazawa University
Chiba University	Osaka City University
Nagoya City University	Nara Medical University
Osaka University	Tokushima University
Wakayama Medical College	Kyushu University
Kobe University	Kagoshima University
Yamaguchi University	Nagasaki University
Kurume University	Nagoya Memorial Hospital

Introduction

Human granulocyte colony-stimulating factor (G-CSF) is an endogenous hematopoietic growth factor that acts on neutrophil and macrophage progenitor cells, specifically accelerating their differentiation and proliferation into mature neutrophils with augmented function [1]. Recent progress in genetic engineering techniques have made possible the large-scale production of this substance and enabled its clinical testing in various types of neutropenia. The human G-CSF used in this study was produced in animal cell cultures by Chugai Pharmaceutical Co., Ltd., Tokyo, using genetic engineering techniques. It is recognized as a protein growth factor whose physicochemical and biological properties are identical to those of natural human G-CSF. Its efficacy and clinical safety have been reported elsewhere [7].

This investigation was a multicenter (Table 1), randomized, controlled study evaluating the use of rG-CSF for the prevention of neutropenia (or leukopenia) induced by chemotherapy in patients with urogenital cancer and for the acceleration of recovery from neutropenia, as well as the safety of rG-CSF use.

Patients and methods

A total of 77 patients were enrolled in this study during the period from April to November 1989. Eligibility criteria for patients were as follows:

Offprint requests to: Toshihiko Kotake, Department of Urology, The Center for Adult Diseases, Osaka, 3-3 Nakamichi 1-Chome, Higashinari-Ku, Osaka, Japan

Table 2. Patient characteristics

	Group A[a]	Group B[b]	Total	χ^2 test
Sex: M	26	32	58	
F	6	6	12	NS
Age (years):				
15–29	7	5	12	
30–49	5	14	19	NS
50–69	17	15	32	
70–79	3	4	7	
Diagnosis:				
Bladder cancer	9	12	21	
Testicular cancer	12	13	25	
Prostatic cancer	3	4	7	NS
Renal pelvic ureteral cancer	7	7	14	
Others	1	2	3	
Performance status:				
0	24	24	48	
1	5	8	13	NS
2	3	6	9	
Chemotherapy regimen				
M-VAC	13	16	29	NS
Others	19	22	41	
Totals		32	38	70

[a] Group A = with rG-CSF
[b] Group B = without rG-CSF
NS, Not significant

Table 3. Evaluation criteria for scoring changes in neutrophil counts

	Score
Duration of neutropenia:	
1. There was no such period or the period was shortened by $\geq$3 days	+1
2. The period was shortened by 0–2 days	0
3. The period was not shortened	−1
Neutrophil (or WBC) count nadir:	
1. Improvement by one grade or more according to the criteria of side effects by the Japan Society for Cancer Therapy	+1
2. No changes in grade	0
3. Worsening of grade	−1
Study day after the start of chemotherapy, through the nadir, until the neutrophil count recovered to $\geq$1,500/mm^3 (or 3,000/mm^3 for WBC):	
1. Nadir of neutrophil count was above >1,500/mm^3 (or >3,000/mm^3 for WBC) or the day of recovery was shortened by $\geq$7 days	+1
2. The day of recovery was shortened by 0–6 days	0
3. The day of recovery was not shortened	−1

a histologically confirmed urogenital cancer, a performance status (PS) of 0–3, adequate myeloid function as demonstrated by neutrophil counts of >1,500/mm^3 and platelet counts of >100,000/mm^3, and an age of between 15 and 80 years. Informed consent was obtained from all patients involved. All subjects must have required at least two cycles of chemotherapy, with the first cycle being used as the observation period. Only patients with a neutrophil count of $\leq$1,000/mm^3 were entered in the second, or combined, phase.

Patients were randomly allocated into either the group A (with rG-CSF) or group B (without rG-CSF) chemotherapy arm of M-VAC (methotrexate, vinblastin, Adriamycin, and cisplatin) [8] and other chemotherapy treatments after their eligibility had been confirmed. Of the 77 patients entered, 7 were excluded as being nonevaluable (3 subjects did not receiv rG-CSF, 2 had inadequate rG-CSF treatment, and 2 violated the protocol with concomitant drugs used); thus, a total of 70 patients consisting of 32 in group A and 38 in group B were evaluable. Characteristics of evaluable patients are shown in Table 2. There were no differences between treatment groups in terms of sex, age, type of cancer, general condition, or types of chemotherapy used.

Patients were stratified according to the chemotherapy used. In patients receiving M-VAC, rG-CSF was given subcutaneously for 14 days starting on day 5 of the chemotherapy cycle; in those treated with other types of chemotherapy, it was given on the day following the completion of chemotherapy.

The evaluation of each patient with regard to the effects of rG-CSF on the prevention of and recovery from neutropenia was performed according to strict evaluation criteria as compared with findings during the observation period (first cycle) as listed in Table 3. That is, the following parameters determined at the second, or rG-CSF, treatment period were compared with those determined during the first cycle: reduction in the duration of neutropenia ($\leq$1,000/mm^3) during which infections are most liable to occur; improvement in side effects based on the classification system of the Japan Society for Cancer Therapy [3] and used for determination of the maximum tolerated dose (MTD) for chemotherapy; and reduction in the recovery time to neutrophil counts of $\geq$1,500/mm^3. Scores for all items were summed to give an overall evaluation for the efficacy of rG-CSF. During the second cycle, the neutrophil count nadir, duration of neutropenia, and neutrophil counts on day 15 after the initiation of chemotherapy were compared between group A (with rG-CSF) and group B (without rG-CSF).

For statistical analysis, the chi-square test (X^2 test) and Wilcoxon's rank-sum test (*U*-test) were used for patient characteristics and for each evaluation parameter, including the overall evaluation, respectively. The unpaired *t*-test was used for comparisons based on absolute neutrophil counts between group A (with rG-CSF) and group B (without rG-CSF).

Results

Treatment response

Comparison of evaluation criteria. In this study, changes in peripheral neutrophil counts were used as the parameters for evaluation of efficacy. However, leukocyte counts were used for the evaluation of patients in whom changes in specific neutrophil counts were not determined as scheduled. These results are shown in Table 4.

Duration of neutropenia: The duration of neutrophil counts of $\leq$1,000/mm^3 (leukocyte count of $\leq$2,000/mm^3) was compared between the first and second treatment cycles for each patient. Duration of neutropenia was reduced by $\geq$3 days in 87.5% of group A patients (with rG-CSF), whereas it was not shortened in 44.7% of group B patients (without rG-CSF). Thus, the administration of rG-CSF significantly reduced the duration of neutropenia as determined by comparison of groups A and B (P <0.001).

Neutrophil nadir: The neutrophil nadir was graded according to the classification system of the Japan Society for Cancer Therapy [3], and changes in grade were compared between the first and second treatment cycles for each patient. Group A showed an improvement by 59.4% and group B, by 21.1%; the improvement in grading in group A was significantly greater than that in group B (P <0.01).

Table 4. Score for each parameter of evaluation criteria for changes in neutrophil (or WBC) counts

	Score			Impossible to judge	Total	*U*-test
	1	0	–1			
Duration of neutropenia (or leukopenia):						
Group A (with rG-CSF)	28 (87.5)	2 (6.3)	2 (6.3)	–	32	
Group B (without rG-CSF)	13 (34.2)	8 (21.1)	17 (44.7)	–	38	P <0.001
Neutrophil (or WBC) count nadir:						
Group A	19 (59.4)	12 (37.5)	1 (3.1)	–	32	
Group B	8 (21.1)	26 (68.4)	4 (10.5)	–	38	P <0.01
Day of recovery to neutrophil counts of $\geqslant$1,500/mm^3 (or $\geqslant$3,000/mm^3 for WBC):						
Group A	28 (87.5)	4 (12.5)	0 (0)	0 (0)	32	
Group B	6 (15.8)	14 (36.8)	12 (31.6)	6 (15.8)	38	P <0.001

Numbers in parentheses represent the percentage of the total number of patients

Table 5. Overall evaluation of changes in neutrophil (or WBC) counts

	Rank				Total	*U*-test
	3	2	1	0		
Group A (with rG-CSF)	19 (59.4)	7 (21.9)	2	4	32	
	[81.3]					
Group B (without rG-CSF)	3 (7.9)	5 (13.2)	4	26	38	P <0.001
	[21.1]					
Totals	22	12	6	30	70	

Numbers in parentheses represent the percentage of the total number of patients; numbers in square brackets represent the accumulated percentage

Recovery time: The time (study days) required for neutrophil counts to reach $\geqslant$1,500/mm^3 (leukocyte count of $\geqslant$3,000/mm^3, after the start of each chemotherapy cycle was compared between the first and second cycles for each patient. In 87.5% of group A patients, the neutrophil counts did not show a decrease to $\leq$1,500/mm^3 and the time to recovery was reduced by $\geqslant$1 week. In comparison, this rate was only 15.8% for group B and the recovery time tended to be prolonged. There was a statistically significant difference in the recovery time between groups A and B (P <0.001). When the neutrophil counts did not recover in six patients, the subsequent chemotherapy cycle was commenced at the investigators' discretion.

Overall evaluation: Each patient was numerically evaluated for the above three parameters and the results were scored for determination of the effects of rG-CSF on the

Table 6. Comparison of changes in neutrophil counts

	Type of therapy	Group A	Group B	Unpaired *t*-test
		Mean ± SD [Study day]	Mean ± SD [Study day]	
Neutropenia duration (days)	M-VAC	1.1±1.7	7.1± 6	**
	Others	0.9±1.3	6.4± 3.3	***
	Total	1 ±1.5	6.7± 4.6	***
Nadir of neutrophil count (/mm^3)	M-VAC	2,104 ±1,881 [11.8±3.6]	436 ±458 [17.7±4]	* [***]
	Others	1,987 ±2,428 [11.5±2.8]	375 ±432 [15 ±2.9]	* [***]
	Total	2,034 ±2,192 [11.6±3.1]	401 ±438 [16.1±3.6]	*** [***]
Neutrophil count on day 15 (/mm^3)	M-VAC	15,632 ±7,232	942 ±687	***
	Others	10,702 ±8,291	652 ±861	***
	Total	12,674 ±8,111	768 ±796	***

* P <0.05, ** P <0.01, *** P <0.001

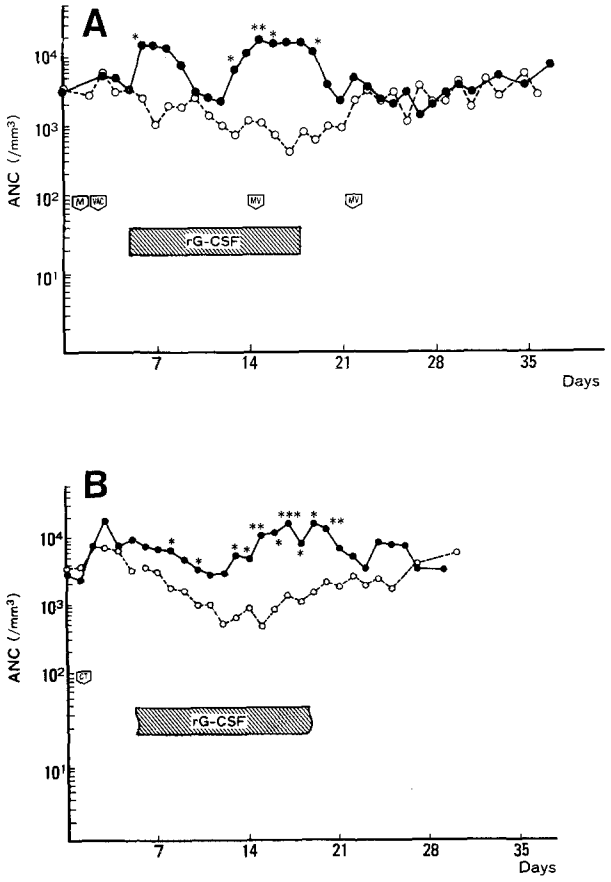

Fig. 1 A, B. Changes in neutrophil counts after the initiation of chemotherapy in the second cycle. **A** Neutrophil count in patients treated with M-VAC. ●——●, Group A (n = 2–12); ○- - - -○, group B (n = 2–14). **B** Neutrophil count in patients treated with chemotherapy other than M-VAC. ●——●, Group A (n = 2–16); ○- - - -○, group B (n = 2–18). * P <0.05, ** P <0.01, *** P <0.001

prevention of neutropenia and the acceleration of recovery. As shown in Table 5, 59.4% of group A and 7.9% of group B were given a rating of +3, and a difference between treatment given to groups A vs B was observed in the number of ranked cases (P <0.001), indicating statistical significance in favor of rG-CSF.

Comparison of changes in neutrophil counts. Patients were stratified by type of chemotherapy into M-VAC and "other" chemotherapy. Excluding 5 subjects for whom neutrophil counts were not adequately observed, a total of 65 patients were evaluated for the effect of rG-CSF on neutrophils. Group A comprised 12 patients who received M-VAC and 18 who were given other chemotherapy, and group B consisted of 15 subjects who were treated with M-VAC and 20 who received other chemotherapy. The results are shown in Table 6.

Duration of neutropenia: The mean duration of neutropenia at neutrophil counts of $\leq$1,000/mm^3 was 1$\pm$1.5 days for group A and 6.7$\pm$4.6 days for group B, indicating that this parameter was significantly reduced in group A (P <0.001). Significant differences were also observed in the M-VAC and other chemotherapy groups. Neutrophil counts did not decrease to $\leq$1,000/mm^3 in 16 cases (53.3%) in group A as compared with 3 cases (8.6%) in group B, and the difference between these groups was statistically significant (P <0.001).

Neutrophil nadir: The mean neutrophil nadir was 2,034$\pm$2,192/mm^3 in group A and 401$\pm$438/mm^3 in group B, and this difference was again significant (P <0.001). The mean study day before the onset of the neutrophil nadir was significantly reduced to 11.6$\pm$3.1 days in group A as compared with 16.1$\pm$3.6 days in group B (P <0.001). These results suggest that the administration of rG-CSF accelerated the proliferation of neutrophil progenitors in the bone marrow, resulting in an acceleration of the recovery of counts in the peripheral blood.

Neutrophil count on day 15: The neutrophil counts on day 15 were examined to evaluate the feasibility of subsequent chemotherapy treatment as scheduled. The average neutrophil count was 12,674$\pm$8,111/mm^3 for group A and 768$\pm$796/mm^3 for group B, with this difference being statistically significant (P <0.001). In M-VAC chemotherapy, methotrexate and vinblastine are scheduled to be given on day 15; however, it is reported that the administration of these drugs is often delayed due to myelotoxicity, especially neutropenia. If neutrophil counts return to normal levels by this time with the administration of rG-CSF, chemotherapy can be continued as scheduled. Changes in neutrophil counts for the second cycle, after the initiation of chemotherapy stratified into M-VAC and other treatment, are shown in Fig. 1.

Side effects

Details of patients showing adverse clinical symptoms and/or abnormal laboratory values that were attributed to the administration of rG-CSF included one subject with fever and diarrhea. In this patient, rG-CSF was discontinued and the fever abated on administration of antipyretics, with the diarrhea disappearing subsequently. Another patient with fever recovered after the drug had been discontinued and antibiotics had been given. Abnormal laboratory values were observed in six patients, including one instance of elevated GOT and GPT, one of decreased cholinesterase levels, four cases of elevated lactic dehydrogenase values, three of elevated Al-P, one instance of decreased total protein, one case of increased gamma-glutamyl transpeptidase, and one of abnormality in urinary protein levels, although all of these episodes were transient.

Discussion

To date, G-CSF has been reported to be the most potent drug available to prevent neutropenia and accelerate neutrophil recovery, and there have been several reports of its use in cancer chemotherapy. Bronchud et al. [2] gave G-CSF at a dose of 1–40 μg/kg per day by 24-h intravenous infusion in combination with chemotherapy (Adriamycin, ifosfamide, and etoposide) for small-cell

lung cancer. Morstyn et al. [5] gave G-CSF b. i. d. to patients with metastatic cancer at a dose of 1–60 μg/kg per day by intravenous drip over 20–30 min in treatment with melphalan. In both of these studies, a remarkable recovery from neutropenia was demonstrated. In the treatment of urogenital cancer, Gabrilove et al. [4] investigated the effects of G-CSF given in combination with M-VAC chemotherapy to patients with transitional-cell carcinoma, and subcutaneous rG-CSF was reported to be effective against neutropenia caused by conventional combination chemotherapy (mitomycin C, vindesine, and cisplatin) for non-small-cell lung cancer [6]. Based on these results, the effect of rG-CSF on neutropenia caused by chemotherapy in urogenital cancer patients was investigated during the second cycle of treatment by comparing the rG-CSF treatment group (group A) with a control group (group B) in patients who had developed neutropenia during the first cycle.

In consideration of the types of drug used and the intervals between doses of chemotherapy, patients were stratified into M-VAC and "other" chemotherapy groups, and the study was conducted after patients had been randomly assigned to rG-CSF treatment and control groups. The parameters used for the evaluation of efficacy were the neutrophil nadir (lowest counts), the duration of neutropenia, and the time required for recovery from neutropenia. After these items had been scored, evaluations of each parameter as well as overall analyses were performed. Based on these evaluation criteria, high effectiveness was considered to imply the feasibility of an increase in the dose and/or a reduction in the cycle interval for the chemotherapeutic agent that caused the dose-limiting neutropenia.

Analysis of the prevention or moderation of neutropenia revealed that the nadir was elevated and the number of patients with neutrophil counts of $\leq 1{,}000/mm^3$ was decreased in group A (with rG-CSF). The duration of neutrophil counts of $\leq 1{,}000/mm^3$ in patients treated with M-VAC was 1.1 days, which was significantly reduced as compared with the 7.1-day duration observed in group B. With respect to the acceleration of recovery from neutropenia, group A showed earlier recovery of neutrophil counts to $\geq 1{,}500/mm^3$ as compared with group B. Especially in patients receiving M-VAC treatment, the mean neutrophil count on day 15 was $15{,}632/mm^3$ in group A as compared with $942/mm^3$ in group B. Based on these counts, it was clinically judged that subsequent chemotherapy could be given without delay on day 15. In patients treated with other chemotherapy, the duration of neutropenia was reduced by 1 week, suggesting that the initiation of subsequent chemotherapy could be expedited.

Gabrilove et al. [4] infused G-CSF intravenously at a dose of 3–60 μg/kg per day during M-VAC therapy in patients with transitional-cell carcinoma and compared the dosing phase with the non-dosing phase in the same patients. These authors reported that the duration of neutropenia ($<1{,}000/mm^3$) was 2.7 days during the non-dosing period, 0.25 day during the dosing period, and 2.3 days in patients who received radiotherapy [4]. Since neutrophil counts were monitored for only the first 14 days after the start of chemotherapy, these results were considered to be comparable with ours. Moreover, the changes in neutrophil counts were related to dose levels in the present study. The subcutaneous dose we used was considered to correspond to an intravenous infusion of 30–60 μg/kg per day when neutrophil counts were compared on day 15. Subcutaneous rG-CSF administration, which gave results comparable with those obtained after intravenous infusion at higher doses, was considered to be appropriate in these cases.

The present study found that the administration of rG-CSF resulted in the moderation or prevention of the development of neutropenia during chemotherapy for urogenital cancer and in a decrease in the duration of neutropenia.

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