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Macrophage Colony-stimulating Factor Prevents Febrile Neutropenia Induced by Chemotherapy

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There are very few studies describing the preventive effect of macrophage colony-stimulating factor (M-CSF/CSF-1) on chemotherapy-induced infection. In this study, we evaluated the changes in superoxide anion production by granulocytes before and after chemotherapy in ovarian cancer patients and investigated the preventive effect of M-CSF on chemotherapy-induced febrile neutropenia. Three courses of chemotherapy [paclitaxel 180 mg/m^2 and carboplatin (area under the curve; AUC 5)] were administered to 32 ovarian cancer patients, and seven patients presented febrile neutropenia. In the 25 afebrile patients, the percentage of superoxide anion production by granulocytes was significantly decreased from 86.5±7.7 (%) to 75.1±8.8 (%) at day 7 and 71.0±6.3 (%) at day 14 without administration of CSF. However, in the patients who presented febrile neutropenia, it was more severely decreased from 86.8±6.8 (%) to 60.0±9.9 (%) at day 7 and 56.8±5.0 (%) at day 14 without administration of CSF. When M-CSF was administered to all patients in the next course with the same dose of chemotherapy, the incidence of febrile neutropenia was significantly decreased (P=0.0195), and the duration of fever (≥38.0°C) and high serum C-reactive protein (CRP) (≥2.0 mg/dl) were also significantly shortened (P=0.0023, P=0.0051). Moreover, in these M-CSF-treated patients, the percentage of superoxide anion production by granulocytes was maintained at the level before chemotherapy. These findings indicate that severe impairment of granulocyte function leads to febrile neutropenia, and that M-CSF reduces the incidence of febrile neutropenia by maintaining or improving granulocyte function.

Key words: Chemotherapy — Febrile neutropenia — M-CSF — CSF-1 — Granulocyte function

Dose intensity in chemotherapy has recently been a focus of interest, and the importance of strategies for the prevention of infection in patients under myelosuppression is increasing.[1–8] In current clinical practice, administration of granulocyte colony-stimulating factor (G-CSF) is commonly prescribed for patients with chemotherapy-induced granulocytopenia. G-CSF significantly shortens the period during which the neutrophil counts are less than 500/μl after chemotherapy, but the prevention of infection by G-CSF is controversial.[9–15] Macrophage colony-stimulating factor (M-CSF/CSF-1) has also been reported to shorten the duration of neutropenia and to prevent the onset of infection; this agent slowly improves the hematopoietic cell system, including granulocyte and platelet counts impaired by chemotherapy.[16–22]

From the viewpoint of infectious disease prevention, granulocyte function is one of the most important factors, and suppression of this function may lead to serious infection. The influence of G-CSF on granulocyte functions in chemotherapy-induced neutropenia has recently been reported,[23–27] but little is known about the influence of M-CSF on neutrophil functions in chemotherapy-induced neutropenia. Clinically, it is important not only to increase neutrophil counts in patients after chemotherapy, but also to prevent the onset of infection in these patients by administering CSFs. Therefore, we investigated the preventive effect of M-CSF on chemotherapy-induced febrile neutropenia, and the effect of M-CSF on granulocyte function in such a condition.

MATERIALS AND METHODS

Patients We studied thirty-two patients with ovarian cancer who were treated in Toyama Medical and Pharmaceutical University Hospital between June, 1997 and May, 2001. Their characteristics are shown in Table I. The mean age of all thirty-two patients was 47.9±14.6 (median, 51; range, 15–63) years. The distribution of the International Federation of Gynecology and Obstetrics (FIGO) clinical stages was Ic, 14; IIc, 1; IIIc, 13; IV, 4. The Eastern Cooperative Oncology Group (ECOG) performance status of the patients was grade 0 to 1 in all cases. We defined febrile neutropenia that presented grade III to IV neutropenia as an absolute neutrophil count in the peripheral blood of less than 1000/μl, fever over 38.0°C, and serum C-reactive protein (CRP) over 2.0 mg/dl. According to this definition, seven of thirty-two patients presented febrile neutropenia. In terms of patients' characteristics, there was no significant difference between the two groups.

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Informed consent was obtained from each patient before treatment.

Treatment The treatment profile is shown in Fig. 1. Primary management consisted of radical cytoreductive surgery, including total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, pelvic and para-aortic lymphadenectomy and tumor debulking, which was followed in all cases by at least three consecutive courses of chemotherapy at the same dose [paclitaxel 180 mg/m^2 and carboplatin (area under the curve; AUC 5)] with a 3 to 4-week interval. In patients who presented chemotherapy-induced febrile neutropenia at first or second chemotherapy, M-CSF (8 million units) was infused over 60 min once daily from day 1 to day 7 beginning 24 h after chemotherapy during the next course. When the patients presented grade IV neutropenia (neutrophil counts<500/μl), G-CSF (2 μg/kg) was injected subcutaneously and prophylactic antibiotics were injected intravenously until the neutrophil count exceeded 2000/μl or white blood cell count exceeded 5000/μl. In 25 afebrile patients, M-CSF (8 million units) was prepared in the third course. Then, we examined superoxide anion production by granulocytes before (day 0) and after chemotherapy (day 7) and also examined whether M-CSF shortened the duration of fever (≥38.0°C) and high serum CRP (≥2.0 mg/dl) in the next course. In addition, the following parameters were also examined to assess the efficacy of M-CSF: duration of grade III neutropenia (neutrophil counts<1000/μl), neutrophil nadir, platelet nadir, total dose of G-CSF, total dose of antibiotics and total dose of immunoglobulin. To clarify the preventive effect of M-CSF on chemotherapy-induced febrile neutropenia, we compared the incidence of febrile neutropenia, and duration of febrile days and high serum CRP between the M-CSF-untreated group (the group to which M-CSF was not administered in the second course) and the M-CSF-treated group (all patients to whom M-CSF was administered in the third course).

Granulocyte function As a measure of granulocyte function, we determined the production of superoxide anion by

Table I. Patients' Characteristics

		Afebrile patients	Febrile neutropenia patients
Case number		25	7
Age	mean±SD	47.7±14.4	54.1±6.0
	median	56	51
Performance status	0	22	6
	1	3	1
FIGO stage	Ic	11	3
	IIc	1	0
	IIIc	10	3
	IV	3	1

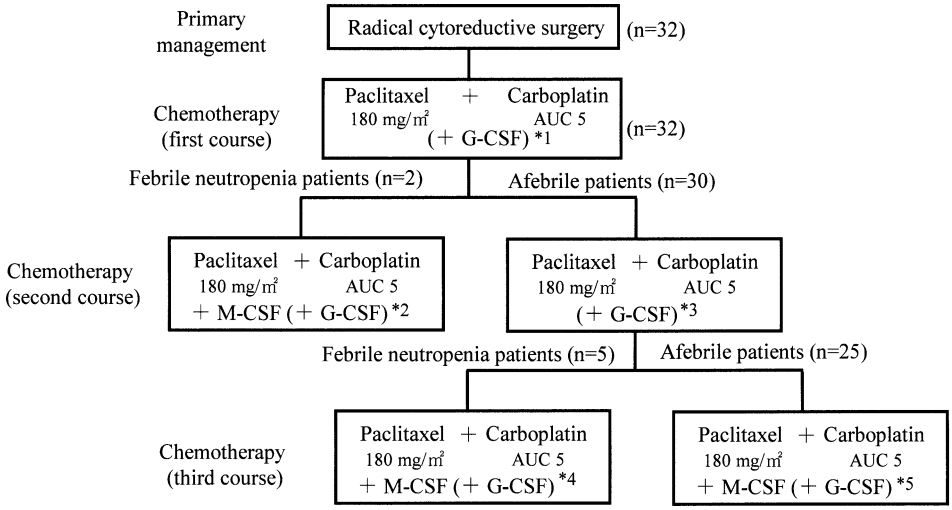

Fig. 1. Treatment profile. Primary management consisted of radical cytoreductive surgery, followed by at least three consecutive courses of the same dose and regimen of chemotherapy [paclitaxel 180 mg/m^2 and carboplatin (AUC 5)]. For those patients who presented chemotherapy-induced febrile neutropenia, M-CSF (8 million units) was prepared during the next course. In afebrile patients, the same dose of M-CSF was prepared in the third course. When the patients presented grade IV neutropenia (neutrophil counts<500/μl), G-CSF (2 μg/kg) and prophylactic antibiotics were prepared until neutrophil counts exceeded 2000/μl or white blood cell counts exceeded 5000/μl. *1: G-CSF was administered to 12 out of 32 patients. *2: G-CSF was administered to 1 out of 2 patients. *3: G-CSF was administered to 13 out of 30 patients. *4: G-CSF was administered to 2 out of 5 patients. *5: G-CSF was administered to 5 out of 25 patients.

granulocytes, which was measured by flow cytometry as previously described by Bass *et al*.[28] Briefly, nonfluorescent [2,7]-dichlorofluorescein was incorporated by the cells and oxidized to fluorescent material by active oxygen produced by PMA stimulation, then the level of fluorescence was measured by flow cytometry. Using all measured neutrophils as the population, the percentage of positive cells was determined.

Statistical analysis Levels of significance were determined using the paired *t* test, unpaired *t* test or χ^2 test. The criterion of significance was defined as $P<0.05$.

RESULTS

At least three courses of chemotherapy were administered to thirty-two patients. Seven of thirty-two patients presented febrile neutropenia (Fig. 1, Table I). In all seven cases, blood and urine culture tests were negative.

In the afebrile patients, the percentage of superoxide anion production by granulocytes was significantly decreased from 86.5±7.7 (%) to 75.1±8.8 (%) at day 7 and 71.0±6.3 (%) at day 14, respectively ($P<0.0001$, $P<0.0001$) (Fig. 2A). Eight patients were administered G-CSF between day 7 and day 14. The percentage of superoxide anion production by granulocytes in the afebrile patients at day 14 was 71.0±6.3 (%), and there was no significant difference between the G-CSF treated (68.4±5.6) and untreated patients (72.3±6.4) ($P=0.1535$).

In patients who presented febrile neutropenia, the percentage of superoxide anion production by granulocytes was more severely decreased from 86.8±6.8 (%) to 60.0±9.9 (%) at day 7 and 56.8±5.0 (%) at day 14 ($P=0.0003$, $P<0.0001$). To all of these seven patients, G-CSF was administered between day 7 and day 14. However, the percentage of superoxide anion production by granulocytes was not improved by G-CSF treatment (Fig. 2B). In these seven patients, the percentage of superoxide anion production by granulocytes before chemotherapy was similar to that in the afebrile patients. However, the degree of the decrease was significantly larger than that in the afebrile patients ($P=0.0024$). There were no significant differences in terms of the mean (±SD) nadir of the neutrophil counts (376±162 versus 353±103, $P=0.8943$) and the duration of the grade IV neutropenia (3.5±2.4 days versus 4.3±2.5 days, $P=0.5373$) between the afebrile patients and the patients who presented febrile neutropenia, respectively.

In patients who presented febrile neutropenia, the median number of days with fever over 38.0°C was 7.4±4.1 (median, 7; range, 2–13), and the mean number of days with serum CRP greater than 2.0 mg/dl was 6.6±4.2 (median, 6; range, 2–13). When M-CSF was

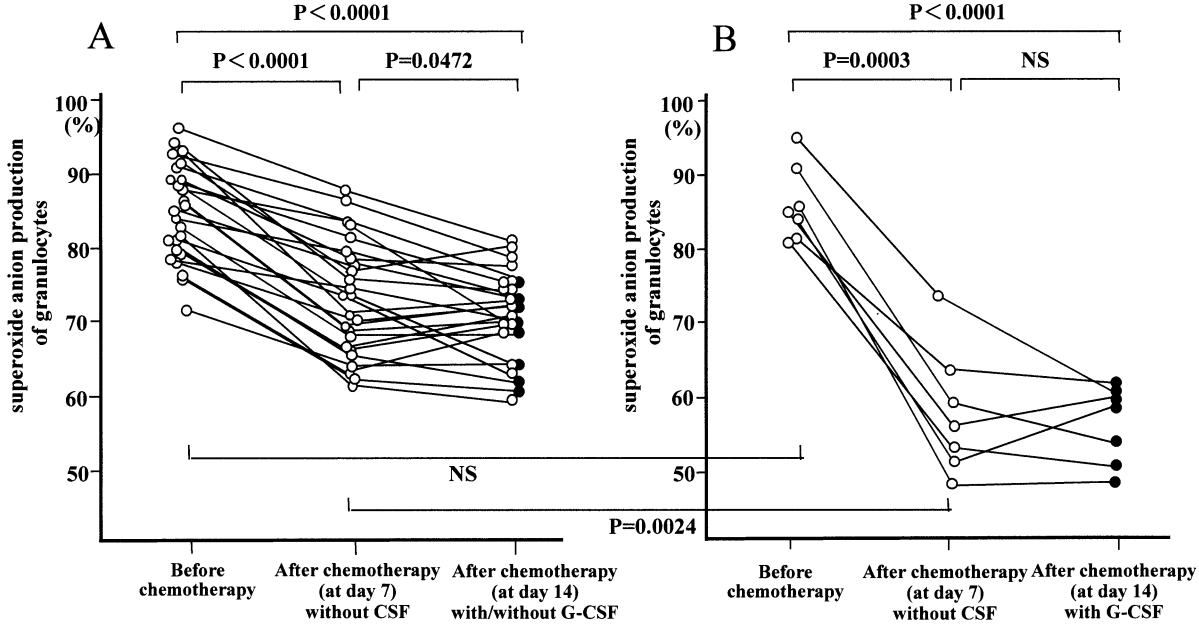

Fig. 2. Superoxide anion production by granulocytes without administration of CSFs before and after chemotherapy (at day 7) and with or without administration of G-CSF after chemotherapy (at day 14) in afebrile patients (A) and in patients who developed febrile neutropenia (B). Heparinized venous blood was obtained from patients before chemotherapy and after chemotherapy (at day 7). Percentages of superoxide anion produced by granulocytes were determined by FACS as described in the text. Closed circles indicate the cases to which G-CSF was administered between day 7 and day 14 after chemotherapy.

administered in the next course, the mean number of days with fever over 38.0°C and the mean number of days with serum CRP greater than 2.0 mg/dl were significantly decreased to 2.4±2.0 (median, 2; range, 0–5) (P=0.0093) and 1.1±1.9 (median, 0; range, 0–5) (P=0.0042) compared with those values during the first course, respectively (Fig. 3). In these cases, the percentage of superoxide anion production by granulocytes was 86.0±4.1 (%) at day

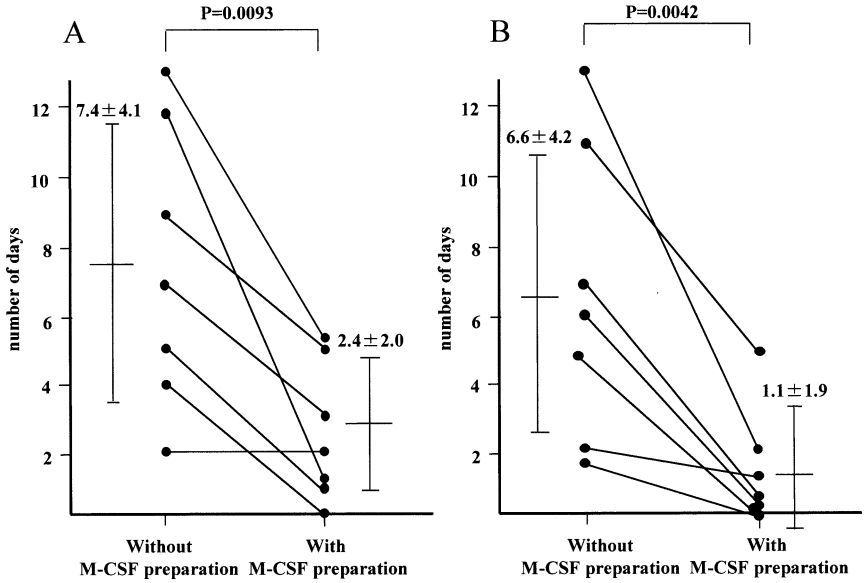

Fig. 3. Effects of M-CSF on the duration of fever over 38.0°C (A) and serum CRP over 2.0 mg/dl (B) in patients who developed neutropenia. Vertical bars indicate means±standard deviations.

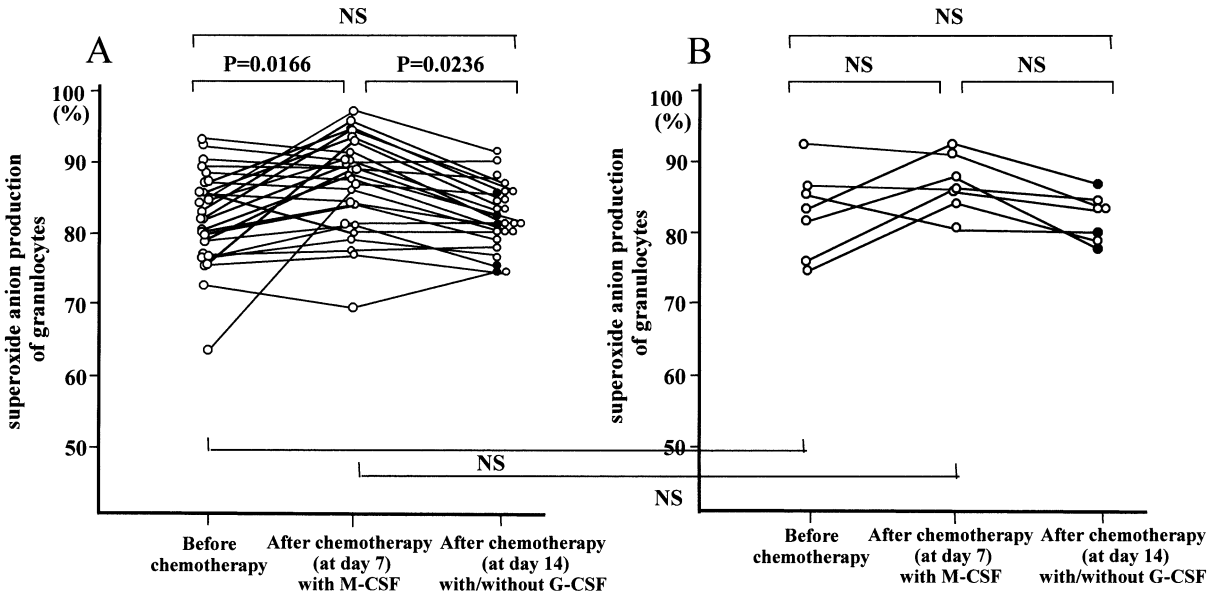

Fig. 4. Superoxide anion production by granulocytes with administration of M-CSF before and after chemotherapy (at day 7) and with or without administration of G-CSF after chemotherapy (at day 14) in the afebrile patients (A) and in the patients who developed febrile neutropenia (B). Closed circles indicate the cases to which G-CSF was administered between day 7 and day 14 after chemotherapy.

7 and 82.6±2.9 (%) at day 14 after chemotherapy, and was maintained at the level before chemotherapy (83.0±5.6) (Fig. 4B). In afebrile patients, the percentage of superoxide anion production was 86.1±6.0 (%) at day 7 and 82.7±4.8 (%) at day 14. The percentage of superoxide anion production at day 7 was significantly improved with administration of M-CSF (P=0.0166) and was maintained at day 14 at the level before chemotherapy, though G-CSF administration was ineffective (Fig. 4A).

In terms of duration of grades III to IV neutropenia, neutrophil nadir and platelet nadir, no significant difference was obtained by administration of M-CSF. However, the total doses of G-CSF, antibiotics, and immunoglobulin were significantly decreased by administration of M-CSF in these seven cases (Table II).

To clarify the preventive effect of M-CSF on chemotherapy-induced febrile neutropenia, we compared the incidence of febrile neutropenia, and duration of febrile days and high serum CRP between the M-CSF-untreated group (the group to which M-CSF was not administered in the second course, n=30) and the M-CSF-treated group (all patients to whom M-CSF was administered in the third course, n=30). Five out of thirty patients in the M-CSF-untreated group presented febrile neutropenia, but no patients in the M-CSF-treated group did so, and there was a significant difference in the incidence of febrile neutropenia between the two group (P=0.0195). In addition, duration of fever over 38.0°C, duration of high serum CRP (greater than 2.0 mg/dl), and the total doses of G-CSF, antibiotics, and immunoglobulin in the M-CSF-treated group were all significantly lower than those of the M-CSF-untreated group. In terms of neutrophil nadir, there was no significant difference (Table III).

Table II. Clinical Data and Supportive Therapy in Seven Febrile Patients with/without M-CSF Treatment

		Without M-CSF	With M-CSF	P value
Neutrophil nadir (/mm^3)	mean±SD	431±252	453±287	NS
Duration of neutropenia	mean±SD	4.1±2.5	3.4±2.3	NS
(<500/mm^3), days	median	4	3	
Duration of neutropenia	mean±SD	12.8±3.8	11.6±4.9	NS
(<1000/mm^3), days	median	13	12	
Platelet nadir (/mm^3)	mean±SD	12.4±6.7	16.2±10.6	NS
Duration of fever	mean±SD	7.4±4.1	2.4±2.0	P=0.0093
(≥38 °C), days	median	7	2	
Duration of high serum CRP	mean±SD	6.6±4.2	1.1±1.9	P=0.0042
(≥2 mg/dl), days	median	6	0	
Total dose of G-CSF	mean±SD	431±187	113±170	P=0.0012
administration (μg)				
Total dose of antibiotics (g)	mean±SD	8.4±2.3	1.9±2.5	P<0.0001
Total dose of γ-Glb[a)] (g)	mean±SD	3.3±3.5	0	P=0.0453

a) γ-Glb: γ-globulin.

Table III. Clinical Data and Supportive Therapy in Patients with/without M-CSF Treatment

		M-CSF-untreated group (n=30)	M-CSF-treated group (n=30)	P value
Incidence of febrile neutropenia		5/30	0/30	P=0.0195
Neutrophil nadir (/mm^3)	mean±SD	624±440	667±519	NS
Duration of fever	mean±SD	2.6±3.0	0.7±1.3	P=0.0023
(≥38°C), days	median	2	0	
Duration of high serum CRP	mean±SD	2.8±4.1	0.6±1.1	P=0.0051
(≥2 mg/dl), days	median	2	0	
Total dose of G-CSF	mean±SD	113±171	35±83	P=0.0297
administration (μg)				
Total dose of antibiotics (g)	mean±SD	2.2±3.3	0.4±1.3	P=0.0067
Total dose of γ-Glb (g)	mean±SD	0.5±1.5	0	P=0.0495

DISCUSSION

Infection related to granulocytopenia during chemotherapy is a critical issue, because it progresses rapidly and causes high mortality. Fever in neutropenic patients is mostly culture-negative. So, it is necessary to start empiric treatment before the culture findings become available, and for this reason, guidelines for treatment were issued by the Infectious Disease Society of America (IDSA).[29] In these guidelines, the criteria for febrile neutropenia are a fever above 38.0°C and grades III to IV neutropenia (absolute neutrophil counts in the peripheral blood of less than 1000/μl). In addition, we added CRP above 2.0 mg/dl to these criteria as a marker of the inflammation state, and treated febrile neutropenia as a state of clinical infectious disease.

In current clinical practice, administration of G-CSF is commonly prescribed to treat granulocytopenia after chemotherapy. Many clinicians consider that infection can be prevented by the granulocyte-increasing effect of G-CSF.[30, 31] However, some authors have found that the administration of G-CSF cannot prevent infection during chemotherapy.[10, 11]

In the present study, we assessed the superoxide anion production of granulocytes as a measure of granulocyte function before and after chemotherapy. In the thirty-two ovarian cancer patients, it was uniformly impaired by chemotherapy at day 7 and day 14 after chemotherapy. However, especially in those patients who presented febrile neutropenia, it was more severely impaired at day 7 after chemotherapy. Although G-CSF was administered to all of these patients between day 7 and day 14 after chemotherapy, the superoxide anion production by granulocytes was not improved. There were no significant differences in terms of nadir of the neutrophil counts and the duration of neutropenia between the afebrile patients and the patients who presented febrile neutropenia. These findings suggest that not the granulocyte count, but rather the severe impairment of superoxide anion production by granulocytes leads to febrile neutropenia. Moreover, the use of G-CSF after the nadir of neutrophil counts did not improve the granulocyte function, even if granulocyte counts were increased.

The administration of M-CSF markedly improved the superoxide anion production of granulocytes at day 7 and day 14 after chemotherapy, and reduced the duration of fever over 38.0°C and high serum CRP greater than 2.0 mg/dl. Furthermore, the total use of G-CSF, antibiotics and immunoglobulin was significantly decreased. This suggests that M-CSF reduces the incidence of chemotherapy-induced febrile neutropenia by improving or maintaining the granulocyte function.

To confirm the preventive effect of M-CSF on chemotherapy-induced febrile neutropenia, we compared the incidence of febrile neutropenia between the M-CSF-untreated group and the M-CSF-treated group. The same outcome, that M-CSF prevents the febrile neutropenia, was clearly seen. We also investigated the effect of G-CSF on the superoxide anion production by granulocytes between the cases to which G-CSF was administered and those to which it was not administered, and confirmed that G-CSF did not significantly affect the superoxide anion production by granulocytes. This finding suggests that G-CSF does not improve the granulocyte function.

In this study, three out of seven patients who presented neutropenic fever were administered G-CSF between day 7 and day 14 after M-CSF administration, so cooperative action of M-CSF and G-CSF might be suggested as one reason for the prevention of febrile neutropenia. However, there was no improvement in granulocyte function in the case of G-CSF administration. This suggests that the improvement of granulocyte function by M-CSF administration leads to prevention of febrile neutropenia. In further clinical trials, G-CSF therapy should be compared with M-CSF therapy with respect to the incidence of febrile neutropenia after chemotherapy.

Since an M-CSF receptor, c-*fms*, is not present on granulocytes, the actions of M-CSF on granulocytes may be indirect. The following action mechanism has been demonstrated *in vitro*: M-CSF enhanced interleukin (IL)-8 production by monocytes in a dose-dependent manner and IL-8 increased the expression of adhesion molecules on granulocytes in healthy adults.[32] A study using samples from healthy adults also reported that M-CSF activated neutrophil function via IL-8.[33] Using samples from chemotherapy-induced myelosuppressive patients, Teranishi *et al.* showed that when M-CSF was added to cultured peripheral blood monocytes, IL-8 levels in the supernatant increased with the concentration of M-CSF. When IL-8 was added to cultured granulocytes, the levels of CD18 expression on granulocytes and superoxide anion production by granuloyctes were significantly increased. These observations suggest that M-CSF enhances the production of IL-8 from monocytes *in vivo*, thereby improving chemotherapy-induced granulocyte dysfunction.[34] In addition, a protective effect of M-CSF against fungal infection via monocytes was reported *in vitro* and *in vivo*.[35–39]

In summary, the present findings suggest that granulocyte function should be improved to prevent infection after chemotherapy. Administration of M-CSF is effective for preventing the dysfunction of neutrophils, resulting in the prevention of febrile neutropenia following chemotherapy.

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