นิพนธ์ต้นฉบับ

Recombinant human granulocyte colony-stimulating factors (rhG-CSF) in primary prevention of chemotherapy-induced neutropenia.

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We reported the use of rhG-CSF in 81 cancer patients after chemotherapy to prevent chemotherapy-induced neutropenia. Glycosylated rhG-CSF (Granocyte®) 2 μg/kg/day or non-glycosylated rhG-CSF (Neupogen®) 5 μg/kg/day was administered subcutaneously 24 hours after chemotherapy until absolute granulocyte count was above 10,000/mm³. Febrile neutropenia developed in 18.7% of all cases (Granocyte® 10.7%, Neupogen® 25%). Duration of antibiotics administration was 7 days. Patients with low neutrophil count (less than 1,000/mm³) was seen in 30% of cases. Median absolute granulocyte count nadir (after chemotherapy) was 960/mm³ (Granocyte® 1,215/mm³, Neupogen® 800/mm³). Median duration of rhG-CSF administration was 10 days. Majority of the patients were able to receive subsequent course of chemotherapy on schedule, and 1% of cases died from febrile neutropenia. Common side effects of rhG-CSF were myalgia (27%), bone pain (4.6%) and fever (4%). rhG-CSF can effectively prevent chemotherapy-induced neutropenia with mild side effects comparable to reports from other trials.

Key words : *Colony stimulating factors, Antineoplastic agents, Neutropenia.*

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รายงานการใช้ rhG-CSF ในผู้ป่วยมะเร็ง 81 ราย หลังได้รับยาเคมีบำบัด เพื่อป้องกันภาวะเม็ดเลือดขาวต่ำจากยาเคมีบำบัด ยาที่ใช้มี 2 ชนิดคือ glycosylated rhG-CSF (Granocyte®) ขนาด 2 µg/กก/วัน และ non-glycosylated rhG-CSF (Neupogen®) ขนาด 5 µg/กก/วัน ฉีดเข้าใต้ผิวหนัง 24 ชั่วโมงหลังการให้ยาเคมีบำบัด จนกระทั่งปริมาณเม็ดเลือดขาว neutrophil มากกว่า 10,000/ลูกบาศก์มิลลิลิตร ในผู้ป่วยที่ได้รับ rhG-CSF 18.7% เกิดไข้ร่วมกับภาวะเม็ดเลือดขาวตกต่ำ(Granocyte® 10.7%, Neupogen® 25%) ระยะเวลาที่ใช้ยาปฏิชีวนะเฉลี่ยคือ 7 วัน ผู้ป่วยที่มีเม็ดเลือดขาว neutrophil ต่ำ (น้อยกว่า 1,000/mm³) พบได้ 30% จำนวนเฉลี่ยของเม็ดเลือดขาว neutrophil ต่ำสุด (หลังจากได้รับเคมีบำบัด) 960/mm³ (Granocyte® 1,215/mm³, Neupogen® 800/mm³) ระยะเวลาเฉลี่ยที่ใช้ rhG-CSF คือ10 วัน ผู้ป่วยส่วนใหญ่สามารถให้ยาเคมีบำบัดได้ตามเวลาในการให้ยาครั้งต่อไป และมีผู้ป่วย 1% เสียชีวิตจากการติดเชื้อขณะที่เม็ดเลือดขาวตกต่ำ ผลข้างเคียงที่พบบ่อยของการใช้ยา rhG-CSF คือ อาการปวดเมื่อยตามตัว (27%) ปวดกระดูก (4.6%) และไข้ (4%) rhG-CSF สามารถป้องกันภาวะเม็ดเลือดขาวต่ำจากยาเคมีบำบัดได้ดีและมีผลข้างเคียงน้อยเช่นเดียวกับในรายงานการศึกษาจากต่างประเทศ

The relationship between the dose of chemotherapy and the antitumor response has been demonstrated in both animal models[1] and in several clinical trials.[2-6] Sub-optimal dosing may adversely affect the outcome of the chemotherapy. Dose reduction or delay of chemotherapy is commonly due to chemotherapy-related toxicities. Chemotherapy-induced neutropenia is by far the most common dose-limiting toxicity in clinical oncology. Marked neutropenia is listed as an acute toxicity of 21 of 33 major antineoplastic agents given by one common schedule of administration.[7] Infection during neutropenic states remains one of the most common causes of treatment-related death in cancer patients who receive chemotherapy, and it causes the delayed treatment of many chemotherapy regimens, and thus de-creases the therapeutic efficacy of cytotoxic regimens.

Granulocyte colony-stimulating factor (rhG-CSF) is a glycoprotein hormone which acts primarily to stimulate proliferation, differentiation, and activation of neutrophils. The advent of recombinant DNA technologies has allowed sufficient production of recombinant human granulocyte-colony stimulating factor in Escherichia coli bacteria or Chinese hamster ovary cells.

In vitro and in vivo studies showed that rhG-CSF could effectively increase the number and functions of neutrophils.[8] With the use of rhG-CSF one might be able to ameliorate and prevent chemotherapy-induced neutropenia allowing the delivery of optimal dose intensity of cytotoxic agents.

Our purpose in this study was to determine the efficacy and safety of rhG-CSF in the prevention of neutropenia caused by chemotherapy in cancer patients.

Methods and Materials

Patients: Eighty one patients with histologically confirmed non-hematologic malignancies were enrolled in the study. Eligibility criteria included a preformance status (Zubrod) scale of less than 3, a life expectancy of at least 3 months, normal hepatic (bilirubin < 2 mg/dl, prothrombin time greater than 70%) and renal functions (creatinine < 2mg/dl), no history of medical illness that precluded participation, no history of autoimmune disease, and normal bone marrow function at the start of chemotherapy (neutrophile count > 1,500/mm^3 and platelet count > 100,000/mm^3).

Study medication: Glycosylated rhG-CSF (Granocyte®) was supplied by Chugai, Japan, as vials of lyophilized powder of 100 or 250 μg/vial. It was reconstituted in 1 ml of aqueous solution at concentration of 100 μg/ml and 250 μg/ml, respectively. The glycosylated rhG-CSF was produced by recombinant DNA technology using Chinese hamster ovary cell lines and containing an N-terminal methionine with O-linked glycosylation. Non-glycosylated rhG-CSF (Neupogen®) was supplied by Amgen Roche, Thousand Oaks, CA, as an aqueous solution at a concentration of 300 μg/ml or 480 μg/ml, respectively. The Escherichia coli expressed rhG-CSF of 18.8 Kd differs from the native molecule in that it contains an N-terminal methionine and no O-linked glycosylation. The specific activity of rhG-CSF is approximately 10^8 μ/mg protein.

Study design: The effectiveness and safety of rhG-CSF were evaluated in a experimental pilot prospective phase II clinical study. Before treatment and study, all patients had their history taken and were given physical examinations including vital signs, performance status, determi-

nation of complete blood count including differential, and platelet counts, urinalysis, liver and renal function tests. The rhG-CSF was administered subcutaneously once daily 24 hours after completion of the last dose of chemotherapy. Glycosylated rhG-CSF was given as a 2 μg/kg/day dose and non-glycosylated rhG-CSF at 5 μg/kg/day. Each patient received only the same type of rhG-CSF for the whole study. The treatment was continued until absolute nadir granulocyte count (ANC) >10,000/mm^3 and beyond granulocyte nadir period. The toxicities the of rhG-CSF were assessed at least once a week. Complete blood counts were performed at least thrice weekly until completion of the rhG-CSF therapy and then once weekly thereafter. Renal and liver function tests were performed monthly.

Results

Eighty one patients were enrolled in the study which had a total of 192 rhG-CSF treatment of 192 cycles. Glycosylated rhG-CSF was given to 40 patients for 84 cycles whereas non-glycosylated rhG-CSF was used in 41 cases for 108 cycles. The median age of the patients in the two groups was 51 (range 17-80), and 54 (range 24-78), respectively. Majority of the patientss had a performance status of 1 (55.5%). One case of the glycosylated rhG-CSF group and 5 cases of the non-glycosylated rhG-CSF group received chemotherapy concomittantly with radiation. Fifty four cases (66.7%) had received no prior chemotherapy or radiation. The mean neutrophils count for the two groups prior to the study was 9,150/mm^3 and 9,230/mm^3, respectively (Table 1.) The diagnosis and chemotherapy regimens shown in Table 2.

Both forms of rhG-CSF prevented chemotherapy-induced neutropenia in most of the patients treated. Median absolute nadir granulocyte count was 1,215/mm^3 (range 10-38,700/mm^3) in glycosylated rhG-CSF group, and non-glycosylated group 800/mm^3 (range 20-30,000/mm^3) respectively (Fig I). Median duration of rhG-CSF treatment was comparable in both groups, 9 versus 10 days. Febrile neutropenia occurred in 36 treated cycles from total cycles (18.7%); 9 episods (10.7%) of the glycosylated rhG-CSF group, and 27 episodes (25%) of the non-glycosylated rhG-CSF group. Most of the patients with febrile neutropenia, empirical antibiotics consisting of ceftazidime and amikacin were given promply after septic work up in 27 of 36 cycles (75%). Those who had an obvious source of infection received appropiate antibiotics according to suspected organism. Antibiotics were changed according to the results of culture and in recation to the sensitivity of the infectious agent obtained. For the patients with culture-negative febrile neutropenia, empirical antibiotics were continued until recovery from the neutropenia (AGC >1,000/mm^3) and until they were afebrile for over 72 hours. The same guideline was also applied to those patients with known infectious causes and received specific antibiotics. Etiology of infection from hemoculture was detected in 1 and 6 cases of patients treated with glycosylated and non glycosylated rhG-CSF, respectively (Table 5). The median duration of antibiotic therapy was 7.5 days (range 7-11) in the glycosylated rhG-CSF group, and 7 days (range 1-13) in the non-glycosylated group. rhG-CSF treatment in cases of febrile neutropenia was increased to 4 μg/kg/day for the glycosylated rhG-CSF

Table 1. Patient Characteristics

Patient characteristics	Glycosylated rhG-CSF	Non-glycosylated rhG-CSF	Total
Total No. of patients	40	41	81
Total No. of cycles	84	108	192
Age (years)			
Median 51	54	52	
Range17-80	24-78	17-80	
Sex			
Male 24	(60%)	21(51.2%)	45(55.6%)
Female 16	(40%)	20(49.8%)	36(44.4%)
Performance status			
0	5(12.5%)	9(22%)	14(17.3%)
1	23(57.5%)	22(53.6%)	45(55.5%)
2	12(30.0%)	10(24.4%)	22(27.2%)
Concomittant chemoradiation	1	5	6
Previous treatment			
with chemotherapy	6(15%)	3(7.4%)	9(11.1%)
with radiotherapy	2(5%)	4(9.7%)	6(7.4%)
with chemotherapy and radiotherapy	4(10%)	8(19.5%)	12(14.8%)
No previous treatment	28(70%)	26(63.4%)	54(66.7%)
Hematologic Pretreatment			
Values (/mm^3): (x ±SD)			
WBC9,150(±4,429)	9,230(±4,647)	9,215(±4,542)	
Neutrophils	7,090(±4,070)	7,100(±4,560)	7,109(±4,346)
Platelets	313,000(±166,707)	329,000(±162,410)	321,378(±164,579)

and 10 μg/kg/day for the non-glycosylated rhG-CSF group until recovery from the febrile neutropenia.

The median rhG-CSF treatment was 9 days (range 5-15 days) in the glycosylated rhG-CSF group, and 10 days (range 5-18 days) in the non-glycosylated rhG-CSF group. The overall mortality rate from chemotherapy-induced febrile neutropenia was 1% despite rhG-CSF prophylaxis. Two cases of the non-glycosylated rhG-CSF group died from febrile neutropenia. Both of them were from non-small cell lung

cancer, one received Cisplatin and VP-16 and the other received Carboplatin and VP-16 with concomittant radiation.

Toxicities of rhG-CSF given subcutaneously daily for primary prevention of chemotherapy-induced neutropenia were mild and well tolerated. No patient had to discontinue rhG-CSF treatment from the side effects. Despite daily local injections for several days, no significant local sequeles at the injection sites were seen in our study of 192 cycles. The most common side effects of the rhG-CSF treatments were myalgia in 52 of 192 cycles (27%) and bone pain in 9 of 192 cycles (4.6%). Fever was observed in 4% of the cases but it was an asymptomatic low-grade fever and self recovered after several rhG-CSF injections. Only 2 cases developed skin rash but these required no treatment and disappeared after subsequent courses of treatment.

Discussion

Traditionally, protection against chemotherapy associated neutropenia has been modification of the chemotherapy dose or dose delay. The use of rhG-CSF in clinical practice may be an additional supportive measure that can reduce the likelihood of neutropenia and its complications due to chemotherapy. Primary administration of rhG-CSF was shown to reduce the incidence of febrile neutropenia by approximately 50% in the three major randomized trials in adults in which the incidence of febrile neutropenia was greater than 40% in the control group.[9-12] In our series of patients who received various myelosuppressive chemotherapy regimens, among 62 patients who developed febrile neutropenia the mortality rate was 32% in patients who did not receive rhG-CSF treatment for febrile neutropenia and 7.4% in those who were treated with rhG-CSF for infection during the neutropenic

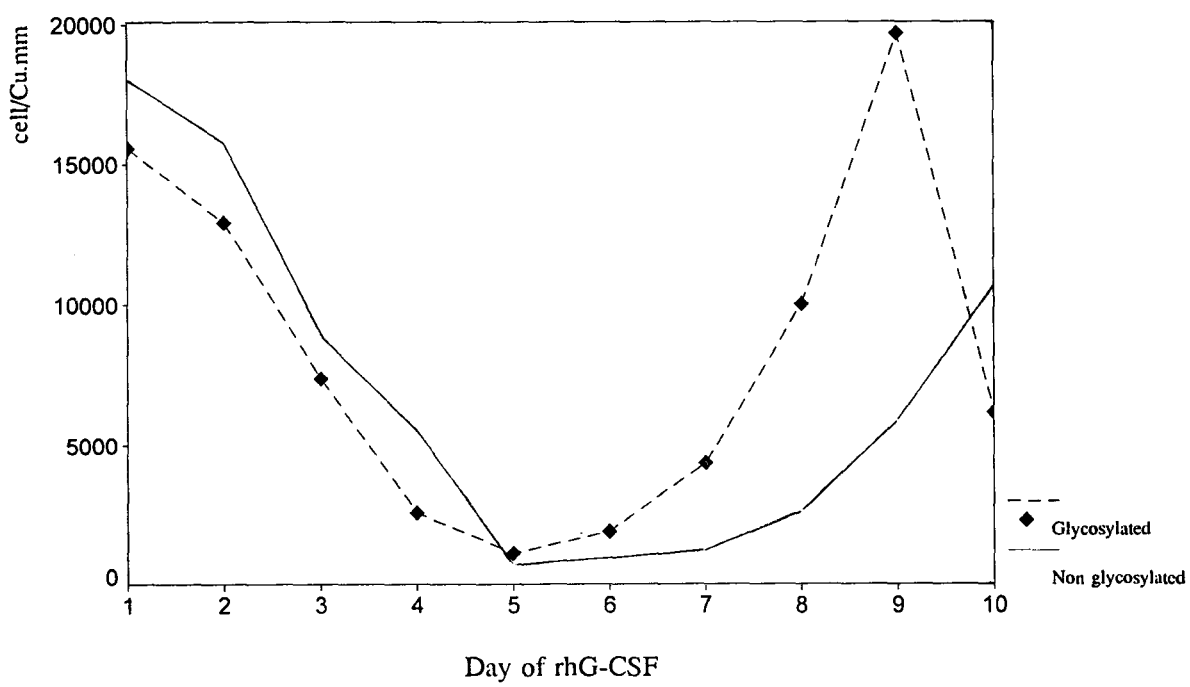

Figure 1. Median absolute granulocyte conunt.

Table 2. Malignancy and Chemotherapy Regimens

Site of Malignancy	Chemotherapy regimens	No. of cycle		
		A*	B**	Total
Head and neck	Ifosfamide	0	6	6
	Carboplatin,Epirubicin,Bleomycin	0	3	3
	Cisplatin, 5FU	3	2	5
	Cisplatin,Epirubicin	0	2	2
	Carboplatin,5FU	0	2	2
	Carboplatin,Etoposide	0	1	1
	Paclitexel	2	0	2
Lung Cancer	Carboplatin,Etoposide	6	13	19
	Cisplatin,VP16	6	5	11
	Cyclophosphamide,Etoposide,Vincristine	0	1	1
	Cyclophosphamide,Etoposide,Epirubicin	0	1	1
	Cisplatin,Epirubicin,Cyclophosphamide	0	6	6
	Paclitaxel	25	19	44
	Paclitexel,Carboplatin	4	0	4
Breast cancer	Cyclophosphamied,Methotrexate,5FU	0	1	1
	Ifosfamide,Mitoxantrone	0	2	2
	Paclitaxel	16	4	20
Stomach	5FU,Etoposide,Leucovorin	0	3	3
Pancrease	Carboplatin,Epirubicin,Leucovorin	0	2	2
Thymoma	Cisplatin,Epirubicin,Cyclophosphamide	0	1	1
Ovary	Ifosfamide	0	1	1
	Paclitaxel	6	4	10
Unknown primary	Carboplatin,Etoposide	0	2	2
	Paclitaxel	0	8	8
Germ cell	Cisplatin,Etoposide,Bleomycin	3	0	3
	Cisplatin,Etoposide, Ifosfamide	1	0	1
	Cisplatin,Vinblastin,Bleomycin,Methotrexate	2	0	2
	Etoposide,Cyclophosphamide,Dactinomycin	2	0	2
Choriocarcinoma	Etoposide,Methotrexate,Cyclophosphamide,Vincristine	0	2	2
Sarcroma	Ifosfamide,Etoposide	0	13	13
	Ifosfamide,Epirubicin	6	4	10
Prostate	Ifosfamide,Epirubicin	1	0	1
Bladder	Cisplatin,5FU,Interferon	1	0	1

Note * Glycosylate rhG-CSF

** Non-glycosylated rhG-CSF

Table 3. Result of rhG-CSF Prophylaxis

	Glycosylated (n=84)	Non-glycosylated (n=108)	Total (n=192)
Febrile neutropenia	9 (10.7%)	27 (25%)	36 (18.7)
Neutropenia (AGC<1,000/mm^3)	30 (35%)	29 (27%)	59 (30%)
Median absolute granulocyte count (AGC) nardir (Range)	1,215 (10-38,700)	800 (20-30,000)	960 (10-38,700)
Duration of treatmetns (days):Median (Range)			
rhG-CSF treatment	9 (5-15)	10 (5-18)	10 (5-18)
Antibiotics treatment*	7.5 (7-11)	7 (3-31)	7 (3-31)
AGC < 1,000/mm*	0 (0-8)	1 (0-9)	1 (0-9)
No. cycles of chemotherapy delay	1	2	3
No. of cycles with dose reduction due to neutropenia	1	1	2
Dead from sepsis	0	2 (1.8%)	2 (1%)

Note *in case of febrile neutropenia

Table 4. Toxicities of rhG-CSF Prophylaxis for Chemotherapy-induced Neutropenia

	Glycosylated (n=84)	Non-glycosylated (n=108)	Total (n=192)
Bone Pain	5(5.8%)	4(3.7%)	9(4.6%)
Myalgia	17(20%)	35(32%)	52(27%)
Fever	3(3.5%)	5(4.6%)	8(4%)
Skin rash	-	2(1.8%)	2(1%)

Table 5. Etiology of Infection in Patient with 36 Febrile Neutropenia

Etiology of infection	glycosylated rhG-CSF (n=9)	non-glycosylated rhG-CSF (n=27)
Hemoculture positive	1(11.1%)	6(22.2%)
- *Klebseilla spp.*	-	3
- *Staphylococcus aureus*	-	2
- *Salmonella spp.*	-	1
- *Enterobacter. spp.*	1	-
Hemoculture negative	8(88.9%)	21(77.8%)
Positive for clinical sites of infection	-	7*
- *Klebseillar spp.*	-	1
- *Streptococcus spp.*	-	1
- *Staphylococcus spp.*	-	1
- *Acinitobacter spp.*	-	1
- *Enterobacter spp.*	-	1
- *Escherichia coli.*	-	2
not found etiology of infection	8	16

*2 cycles, positive from hemoculture and from clinical site

period.[13] In this study, patients who received rhG-CSF prophylaxis after chemotherapy developed febrile neutropenia in 36 of 81 cases (18.7%). Though 30% of the patients treated with rhG-CSF had neutropenia with a median absolute granulocyte count of 960/mm^3, the majority of them received the next cycle of treatment without any delay or dose modification. Only 2 patients (1%) who received primary rhG-CSF prophylaxis died as a sequele of febrile neutropenia. When rhG-CSF was given as a primary prophylaxis, it not only effectively decreased the incidence but also reduced the mortality rate associated with febrile neutropenia.

For clinical use, rhG-CSF is available in 2 forms : a non glycosylated form *(Escherichia coli-derived)* and a glycosylated form (Chinese Hamster Ovary cell-derived). The bacterially derived product consists of 175 amino acids and differs from the endogenous protein in 2 respects; methionine is the terminal amino acid, and it is not glycosylated secondary to being expressed in *E.coli.*[14] The Chinese Hamster Ovary cell-derived glycosylated rhG-CSF has 174 amino acids and 4% carbohydrate located at position threonine 133.[15] Both forms of rhG-CSF show no detectable difference from natural G-CSF in vitro and in vivo in terms of neutrophil stimu-

lation.[16,17] The carbohydrate component of native G-CSF appears to play no part in its biological activity.[16] Although glycosylation per se does not seem to be essential for the biological activity of rhG-CSF, evidence has shown that the glycosylation of the rhG-CSF molecule confers many advantages over non-glycosylation in terms of in vitro stability.[18] and resistance in human serum to protease degradation.[19] In vitro study showed that non-glycosylated rhG-CSF had a biological potency of 30-50% that of glycosylated rhG-CSF as measured by bioassays. Inferior qualitive and quantitative differences in non-glycosylated rhG-CSF stimulated neutrophil colony formation suggests that glycosylation has a potency advantage in vitro.[15]

Both glycosylated and non-glycosylated rhG-CSF at doses and schedule used in this study effectively alleviated severe neutropenia caused by myelosuppressive chemotherapy. A higher incidence of febrile neutropenia (25% versus 10.7%) and highher mortality rate (1.8% versus 0%) was observed in the non-glycosylated rhG-CSF group than in the glycosylated rhG-CSF group. This may be due to more treaments with radiation given concomittantly with chemotherapy in that group (5 versus 1 case), and the difference in chemotherapy regimens. Both of the patients died in the non-glycosylated rhG-CSF group had more myelosuppression as a consequence of chemoradiation.

The toxicities of rhG-CSF were mild and well tolerated. Myalgia was more common in patients treated with non-glycosylated rhG-CSF than glycosylated rhG-CSF (32% versus 20%). Minor drug allergy presented with drug rash was seen in 2 cases who received non-glycosylated rhG-CSF. None of the glycosylated rhG-CSF group patients had drug rash (Table 6).

The American Society of Clinical Oncology has recommended that primary administration of rhG-CSF be reserved for patients expected to experience levels of febrile neutropenia comparable to or greater than 40%. Primary rhG-CSF administration may be exceptionally warranted in patients at higher risk for chemotherapy-induced infectious complications such as preexisting neutropenia due to disease, extensive prior chemotherapy or previous radiation to the pelvis or other sites containing large amounts of bone marrow, a history of recurrent febrile neutropenia while receiving earlier chemotherapy of similar of lesser dose-intensity, or conditions potentially enhancing the risk of serious infection, including decreased immune function, open wounds, or already active tissue infections.[20] The high cost of rhG-CSF has led to concerns regarding its routine use in clinical practice despite its effectiveness in the prevention of chemotherapy-associated neutropenia.[21]

In conclusion, our data suggested that rhG-CSF administration as a primary prevention of chemotherapy-associated neutropenia is effective in the reduction of neutropenia and its complications, and it allows the clinician to maintain dose-intensity of myelosuppressive chemotherapy. Both glycosylated and non-glycosylated rhG-CSF were effective in prevention of neutropenia following myelo suppressive antineoplastic agents in this study. The rhG-CSF was safe and well tolerated by most of the patients in this study.

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