ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY (1998) 16: 43-47

The Use of Lenograstim (Granocyte®) in Chemotherapy for Ovarian Cancer

Anuvat Roongpisuthipong, Issaracha Suphanit, Apisak Luamprapat and Sumrit Senapad

SUMMARY **We have conducted an open, controlled study on the febrile neutropenia effects by Lenograstim (Granocyte®) therapy following cytotoxic chemotherapy of cisplatinum and cyclophosphamide in patients with primary advanced epithelial ovarian cancer. Eligible patients (n=17) were divided into 2 groups receiving a combined chemotherapy of intravenous cisplatinum (70 mg/m^2) and cyclophosphamide (700 mg/m^2) with or without the addition of Lenograstim. Subcutaneous administration of Lenograstim (100 μg/day) for 7 consecutive days was given from day 8 to day 14 of the 3rd to the 5th cycle of chemotherapy in Lenograstim treated patients. After 3 cycles of treatment, Lenograstim treated patients (group 1, n=10) showed a significant improvement in white blood cell (WBC) count as compared with group 2 (control) of 7 patients (p = 0.00002). Group 1 patients also showed an increased C-reactive protein, though of no significance. There were no significant differences among the 2 groups regarding ESR, hematocrit, platelet counts and blood chemistry profiles. This preliminary data encourages more study of the benefits of Lenograstim in the treatment of ovarian cancer.**

Lenograstim (Granocyte®) is a recombinant human granulocyte colony stimulating factor (rhG-CSF). The use of growth factors has revolutionized chemotherapy in the field of hematology and oncology in recent years. It was used as an emerging new treatment modality. It improved morbidity due to fewer incidences of neutropenia and shorter hospital stay, as well as a better tolerance to a dose intensified chemotherapy regimen, due to the ability of the growth factor to mitigate chemotherapy-induced complications. Granulocyte colony stimulating factors (G-CSF) have been used in chemotherapy for ovarian cancer.[1] Ochai[1] observed in his study the significant effects of Lenograstim in patients with ovarian cancer receiving a chemotherapy regimen. Over 90% of patients receiving Lenograstim showed significantly improved neutropenia. In this current study, our primary objective was to study the effect of Lenograstim therapy in increasing patients' white blood cells following cytotoxic chemotherapy with cisplatinum and cyclophosphamide, in patients with primary advanced epithelial ovarian cancer. Lenograstim was used as the study drug of choice due to its overall efficacy of leucocyte glycosylated profile. It has been shown that glycosylation improves the based on cost analysis as the growth factor of choice compared to the nonglycosylated G-CSF formulations.[2-5] Currently Lenograstim is the only growth factor that is glycosylated.

MATERIALS AND METHODS

The study design was an open-labeled, controlled study. Cri-

From the Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.
Correspondence: Anuvat Roongpisuthipong

teria for patient selection included primary advanced ovarian cancer patients of poorly differentiated cell type, stage IV of *FIGO staging* (Growth involving one or both ovaries with distant metastasis) who were between 18-60 years of age, able and willing to give informed consent and willing to be treated with Cisplatinum and cyclophosphamide therapy. Patients who were enrolled into the study were judged to have a life expectancy of 3 months or greater. Patients with advanced epithelial ovarian cancer who were seen at the Department of Obstetrics and Gynecology, Siriraj Hospital underwent staging and screening procedures according to criteria for patient selection prior to receiving chemotherapy. They were treated with at least 5 cycles of combined chemotherapy of intravenous Cisplatinum (70 mg/m^2)and 700 mg/m^2 oral cyclophosphamide (One week for chemotherapy and three weeks pause). Each cycle lasted approximately 3 weeks. After 2 cycles of chemotherapy, they were then divided into 2 groups by self-voluntary decision whether they wanted to receive the additional G-CSF treatment. Ten patients in group 1 were treated with subcutaneous administration of 100 μg/day of Lenograstim from the third course of chemotherapy on day 8 to day 14 for a period of 7 days and were given for 3 consecutive cycles. In group 2 (untreated control), 7 patients received the same chemotherapy regimen as in group 1 but without Lenograstim. Chemotherapy cycles for this group were anticipated to be longer than those in group 1 because these patients did not receive any G-CSF. The parameters that were assessed for Lenograstim efficacy included changes in WBC count, hematocrit (Hct), platelet count, erythrocyte sediment rate (ESR) and C-reactive protein after the G-CSF treatment. Fever index in individual patients was also observed for at least 8 hours during the chemotherapy and Lenograstim treatment in hospital. Unfortunately the patients in the control group refused to stay in the hospital after receiving chemotherapy, therefore, we were unable to collect the fever index of the control group. Blood chemistry including albumin, globulin, SGOT, SGPT, bilirubin, cholesterol, LDH, FBS, BUN, creatinine and uric acid were analysed before and after the treatment in individual patients.

The results of the study were expressed as changes of the parameters, especially the number of white blood cells after the study treatment compared to the initial values before Lenograstim treatment. The normal limits of various parameters used in this study are shown in Table 1. Nonparametric unpaired t-test was employed for statistical analyses and comparison of the changes in the treated and control groups.

RESULTS

The differences of individual WBC count in 2 groups of the patients before and after Lenograstim administration are shown in Table 2. There is a significant increase in white blood cell count in the Lenograstim treated patients (+91.15%) compared with the decrease in white blood cell count in the control group (-6.51%), P = 0.000002. The results demonstrate a statistically significant improvement in the WBC count in the Lenograstim treated group compared with the control group. The mean ESR level in group 1 patients was elevated by 15 mm/hr after the G-CSF treatment as compared with 10.1 mm/hr in the control group (Table 3). At the end of the study (5th chemotherapy cycle), C-reactive protein was found to be positive in 50% of the patients in group 1. Three cases showed a strong evidence of C-reactive protein whereas 2 patients had only one positive test out of 9 tests.

Table 1. Various parameters' normal limits are shown below. The interpretations are higher, lower or within normal ranges

FBS	76-110 mg/dl
Platelets count	140,000-400,000 cells/mm^3
BUN	7-20 mg/dl
WBC count	5,000-10,000 cells/mm^3
Creatinine	0.5-1.5 mg/dl
Severe leukopenia	≤ 200,000 cells/mm^3
Albumin	3.5-5.5 g/dl
Globulin	1.5-3.5 g/dl
Uric acid	2.4-7.0 mg/dl
ESR	0-20 mm/hr
C-reactive protein	≤ 6 mg/l

Higher value represents inflammatory process
Fever index area under the curve, above cut point (37.8°C) mean inflammation

Table 2. Mean difference of WBC count in the two groups before and after Lenograstim administration

Patient	Lenograstim group (%)	Control group (%)	P - value
A	44.7	-17.0	
B	14.3	1.95	
C	-0.65	-7.6	
D	217.8	-18.5	
E	151.1	0.6	
F	127	18.8	
G	66	-23.8	
H	79	-	
I	75.3	-	
J	137	-	
Mean	+91.15	-6.51	0.000002

Table 3. Mean difference before and after Lenograstim administration from the beginning of the 3rd cycle to the end of the 5th cycle

Labs	Lenograstim group	Control group	P-value
ESR	15 mm/hr	10.1 mm/hr	> 0.05
C-reactive protein	5/10 (50%)	1/7 (14%)	
Strong evidence*	3/10 (30%)	-	
Mild evidence**	2/10 (20%)	1/7 (14%)	
Hct	-4.7%	-4%	> 0.05
Platelets	-127,000/mm^3	-36,857/mm^3	> 0.05

*Strong evidence defined as two or more positive C-reactive protein
**Mild evidence defined as one positive C-reactive protein out of nine examinations.

In contrast, only 1 patient out of 7 in the control group showed one positive test out of 9 tests undertaken (Table 3). Table 3 also reveals a nonsignificant de-crease of the hematocrit values after the treatment in both groups. There were no significant changes of platelet count in both groups although the mean reduction of platelet count in group 1 was higher than that in the control group (-127,000/mm^3 in group 1 *vs.* -36,857/mm^3 in group II)(Table 3). Nevertheless 3 cases out of 10 patients in groups 1 and 2 out of 7 cases in the control group showed a slight increase in platelet count. Blood chemistry results of both groups were mostly within normal limits including fasting blood sugar, liver function tests, BUN, creatinine, and LDH. There were no significant changes in all parameters at the end of the study in both groups. However, 1 patient in group 1 as well as 2 patients in group 2 had a slight increase in fasting blood sugar levels at the end of the study.

DISCUSSION

This study was carried out with a small sample size due to strict enrollment criteria as well as the high cost of rhG-CSF. Despite the small sample size, we believe that the investigation under concomitant chemotherapy with Cisplatinum and cyclophosphamide, had sufficient power to evaluate the use of rhG-CSF in patients with ovarian malignancy. Our study revealed that 90% of the patients receiving CSF treatment had a significant elevation (91% increase) of WBC count from the initial baseline. On the other hand, the control group had more than 6.51% reduction in the WBC count compared with baseline values. The significant effects of rhG-CSF in these patients have again confirmed the benefits of the growth factor in this population receiving chemotherapy. This elevation of WBC will reduce the risk of infections in these high risk patients. On the contrary, no such WBC elevation was observed in the control group and would therefore put them at a very high risk from concurrent infection which in turn might increase morbidity and mortality. Hematocrit and platelets were nonsignificantly decreased in both groups. This may be partially explained on the basis that rhG-CSF stimulates only leukocytes and has no effect on the production of red blood cells or the platelets. The ESR and C-reactive proteins were elevated in the growth factor treated group. This may possibly be due to hidden infections that occurred prior to the study period. Although rhG-CSF was given to patients, these

acute phase reactants were elevated at the end of the fifth chemotherapy course compared with the beginning of the third course (start of study period). This elevation was likely related to an inflammatory of infectious process. The fever index is an excellent marker for infections and inflammation. Unfortunately, we were unable to evaluate this data due to noncompliance with the study period requirements of the patients in the control group. However, the data that we were able to collect from group 1 revealed that there was no correlation of fever index to the ESR and C-reactive protein elevations, implying that there were no apparent infections and/or inflammations. The abnormal increase in fasting blood sugar levels in a few patients may be interpreted as undiagnosed cases of diabetes mellitus. One patient in group 1 and 2 patients in the control group had abnormalities that once again could not be clearly explained by correlation to either infections or inflammation as suspected by the acute phase reactant elevations. The effectiveness of appropriate cycles of chemotherapy is very important in treating patients with malignancies. In a study by Fanning *et al.*,[6] it was shown that in 30 women with primary advanced ovarian cancer, G-CSF allowed up to 50% dose escalation of chemotherapeutic agents for more effective chemotherapy. In another study by Nagai *et al.*,[7] they were also able to show that early treatment with G-CSF in patients with ovarian cancer allowed increased intensity of chemotherapy, by using greater doses, or by shortening the interval cycles. Studies of rhG-CSF have shown that it has a direct effect on superoxide generation and neutrophil activity.[8] In one study from Japan,[9] it was shown that G-CSF induced the polarization of neutrophil, which was considered as the initial reaction for chemotaxis. This may have the effect of enhancing the innate defensive properties of the neutrophil. It is important to realize that this property of Lenograstim on the neutrophil may not just be the quantitative improvement of the neutrophil that will benefit the patient, but also the concurrent improvement in neutrophil chemotaxis. There have been some controversies regarding the use of growth factors in cancer.[10] It has been questioned whether the growth factor may have a negative impact in promoting cancer growth. In one study,[11] GM-CSF and G-CSF were evaluated for the growth of established ovarian cancer cell lines as well as primary ovarian cancer cultures over a wide range of pharmacological doses. G-CSF showed no growth stimulating effects in any of the established ovarian cancer cell lines. In fact, there was a growth reduction (>10%) in the OVCAR-3 cell line. However, the same therapeutic doses of GM-CSF caused an increase (>10%) in growth of the tumor cell line. The results suggested that some but not all ovarian cancer cells showed different responses to different growth factors. However, in another study by Saito *et al.*,[12] evaluating 38 different human cancer cell lines ranging from lung, colon, breast, stomach, brain, as well as melanoma revealed that G-CSF had no stimulatory effect on the growth of these tumor cells.

In conclusion, we have shown that rhG-CSF can significantly stimulate leukocyte production in patients with primary advanced epithelial ovarian cancer receiving standard chemotherapy. This will be advantageous to the oncologist in initiating effective chemotherapy cycles without delay occurring due to leukopenia resulting from previous myelosuppressive chemotherapy. Although there exists controversies concerning growth factor use in cancer, we believe that the benefit of using growth factor is significant enough to warrant its use at the current time. The exciting data of peripheral blood stem cell transplant (PBSCT) in conjunction with Lenograstim[13] use for ovarian cancer have given new hope for treatment for this disease and we look forward to further evaluation in this field. As for our study, we recommend further research with a larger sample size to confirm and re-evaluate our findings.

ACKNOWLEDEMENTS

This work was funded by the Siam Pharmaceutical Company. We thank Dr. Niwat Montreewasuwat of Servier (Thailand) Ltd. for revision of the manuscript.

REFERENCE

1. Ochai K, Terashima Y. Chemotherapy and granulocyte colony-stimulating factor in ovarian cancer. Semin Oncol 1994; 21 (Supp 1): 23-8.
2. Nissen C. Glycosylation of recombinant human granulocyte colony-stimulating factor: implications for stability and potency. Eur J Cancer 1994; 30 (Supp 3): S12-4.
3. Nissen C., Dalle Carbonare V., *et al. In vitro* comparison of the biological potency of glycosylated versus non-glycosylated rG-CSF. Drug Invest 1994; 6: 346-52.
4. Rebischung JL, Deletie E, *et al.* Comparative study of filgrastim and lenograstim. Clinical and economic studies. Proc Am Soc Clin Oncol. 1995; 14: 260 AB:713.

5. Ono M. Physicochemical and biochemical characteristics of glycosylated recombinant human granulocyte stimulating factor (lenograstim). Eur J Cancer 1994; (30A Supp 3): S7-11.
6. Fanning J, Hilgers RD. Prophylactic granulocyte colony-stimulating factor allows escalation of chemotherapeutic dose intensity in advanced epithelial ovarian cancer. Gynecol Oncol 1996; 323-7.
7. Nagai N, Takehara K, *et al.* Clinical evaluation of human granulocyte colony-stimulating factor in chemotherapy for ovarian cancer. Hiroshima J Med Sci. 1995; 99-103.
8. Ichinose Y, Hara N, *et al.* Recombinant granulocyte colony stimulating factor and lipopolysaccaride maintain the phenotype of and superoxide generation by neutrophils. Infect Immun 1990; 58: 1647-52.
9. Hiroto M, Kadota JI, *et al.* Studies on defense effects of recombinant human granulocyte colony-stimulating factor (G-CSF) to infections. Induction of neutrophil polarization by G-CSF. J Jpn Assoc Infect Dis 1994; 4: 425-9.
10. Fanning J, Hilgers RD, *et al.* Sequential granulocyte colony stimulating factor increases cisplatin cytotoxicity in human epithelial ovarian cell lines. Gynecol Oncol 1996; 450-3
11. Connor JP, Squatrito RC, *et al. In vitro* growth of colony stimulating fac-tors in ovarian cancer. Gynecol Oncol 1994; 347-52.
12. Saito M, Ozawa S, *et al.* Biological activity of recombinant human G-CSF on the *in vitro* growth of human tumor cell lines. Jpn Pharmacol Ther 1990; 18(Supp 9): 81-7.
13. Menichella G, Pierelli L, *et al.* Low dose cyclophosphamide with cisplatin or epirubicin plus rhG-CSF allows adequate collection of PBSC for autotransplantation during adjuvant therapy for high risk cancer. Trans-plant 1994; 907-12.