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RECOMBINANT HUMAN GRANULOCYTE – MACROPHAGE COLONY – STIMULATING FACTOR THERAPY AND ENDOGENOUS PLASMA GM – CSF, IL – 3, IL – 4 CONCENTRATIONS IN PEDIATRIC PATIENTS WITH SOLID TUMORS*

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Ten pediatric patients with solid tumors and chemotherapy-induced neutropenia were given recombinant human granulocyte-macrophage colony-stimulating factor (rHuGM CSF). The duration of the neutropenic phase was then compared with the results obtained from eight patients also with solid tumors, but not treated with rHuGM-CSF. It was found that rHuGM-CSF treatment significantly decreased the duration of the neutropenic phase. Endogenous plasma GM-CSF, IL-3, and IL-4 levels were also measured in the study group and in healthy children. No significant correlation has been found between plasma GM-CSF concentrations and absolute neutrophil counts. However, IL-3 levels of the neutropenic patients positively correlated with platelet counts. Furthermore, IL-4 concentrations were positively correlated with the GM-CSF level in the same individual. Plasma GM-CSF, IL-3, and IL-4 levels in the neutropenic solid tumor group were found to be significantly higher than those in healthy children. Plasma IL-4 levels were significantly elevated in patients with osteosarcoma as compared to patients with other solid tumors. Although rHuGM-CSF has a half-life of only two to three hours, one day after rHuGM-CSF therapy, plasma GM-CSF levels were found to be higher than initial values. In contrast, plasma IL-4 values decreased significantly after administration of rHuGM-CSF. The probable mechanisms for the changes in cytokine levels are discussed. *Key words: rHuGM-CSF, plasma GM-CSF concentration, IL-3, IL-4, neutropenia, solid tumor.*

Recombinant human granulocyte-macrophage colony-stimulating factor (rHuGM-CSF) is currently used in various neutropenic conditions, both iatrogenic and disease related[1,2]. rHuGM-CSF accelerates neutrophil recovery after chemotherapy, leading to a reduction in the duration of the neutropenic phase and, consequently, antibiotic usage and duration of hospitalization[1-3]. However, little is known about the endogenous hemopoietic growth factor (HGF) response or plasma

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concentrations of some cytokines in neutropenic patients. Plasma levels of granulocyte colony-stimulating factor (G-CSF) and GM-CSF in some pathological conditions were measured and it was reported that changes in the peripheral neutrophil count almost paralleled those in the plasma G-CSF level, but not in the GM-CSF level[4,5].

Besides the suportive role of recombinant colony-stimulating factors (CSFs) had interleukin-3 (IL-3) in cancer therapy, some cytokines were thought to have direct and indirect anti-tumor effects[2,6]. Recent in vitro studies have indicated that IL-4 had anti-tumor activity[2,7,8]. On the other hand, IL-4 has also been found to be an active factor in myelopoiesis[9,10]. In addition, alterations in the immune status of patients with various cancers may result in the release of cytokines in circulation[11]. Some tumor cells have also been found to secrete cytokines, such as the secretion of IL-4 by malignant cells in Hodgkin's disease[12].

This study is composed of two parts. In the first part, the neutrophil response to rHuGM-CSF and the duration of the neutropenic phase in children with solid tumors are evaluated and compared with another group of patients also with solid tumors, but who were not treated with rHuGM-CSF. In the second part, endogenous plasma GM-CSF, IL-3, and IL-4 levels were measured in patients before and after rHuGM-CSF therapy and in healthy children. These plasma levels were compared with each other. The correlations between plasma GM-CSF, IL-3 levels and peripheral blood neutrophil and platelet counts and also between plasma levels of IL-4 and GM-CSF were analyzed. Although rHuGM-CSF has a very short half-life, its effects on post-treatment plasma IL-3 and IL-4 concentrations were investigated.

Material and Methods

Ten patients (6 girls, 4 boys) with different solid tumors in the active period were included in the study. The age of the patients ranged from two to 18 years with a mean 13.9 ± 4.9. The diagnosis was çonfirmed by histopathological findings. Five of ten children had osteosarcoma (osteoblastic type), while three cases were diagnosed with Ewing's sarcoma. Rhabdomyosarcoma and anaplastic astrocytoma were the other solid tumors. The clinical features of these patients are listed in Table I. None of the patients had clinical or laboratory signs or symptoms of a bacterial or fungal infection before or after rHuGM-CSF treatment. rHuGM-CSF (Leucomax, Sandoz), 5 µg/kg, was administered subcutaneously to the 10 patients with neutropenia. The median rHuGM-CSF treatment duration was seven days (range 5-12 days). The treatment was started in eight patients after their chemotherapy (CT) and/or radiotherapy (RT) schedule and in two patients during their CT/RT schedule (Table II). These patients were not transfused with any blood or blood-derived products during rHuGM-CSF treatment.

Table I: Clinical Features of the Patients (CT: Chemotherapy, RT: Radiotherapy)

Patient No/Sex	Age (Year)	Diagnosis	Treatment
1 (F)	16	Osteosarcoma	CT
2 (F)	18	Osteosarcoma	CT
3 (F)	18	Osteosarcoma	CT
4 (F)	15	Osteosarcoma	CT
5 (M)	17	Osteosarcoma	CT
6 (F)	16	Ewing's sarcoma	CT + RT
7 (M)	14	Ewing's sarcoma	CT + RT
8 (M)	16	Ewing's sarcoma	CT
9 (M)	2	Rhabdomyosarcoma	CT
10 (F)	1	Anaplastic astrocytoma	CT

Table II: The Neutrophil Response to rHuGM-CSF Treatment and Side Effects

		Absolute Neutrophil Count (ANC)/mm^3			
Patient No.	Administration of rHuGM-CSF	Before rHuGM-CSF	Time to Rise Above 1000/mm^3 (Day)	Peak Value/Day	Side Effects
1	After CT	744	1	6468 (6th)	No
2	After CT	1400	–	5016 (2nd)	No
3	After CT	900	3	5360 (3nd)	Fever, itching
4	During CT	392	1	7684 (10th)	Thrombocytopenia
5	After CT	1444	–	22320 (6th)	Fever
6	After CT+During RT	700	1	4255 (2nd)	No
7	During CT+RT	200	Never	385 (9th)	Thrombocytopenia
8	After CT	416	5	2500 (5th)	No
9	After CT	300	4	2838 (7th)	No
10	After CT	966	3	2508 (4th)	Fever
Median		722	3	4636 (5.5)	

Ten healthy children aged 10.3 ± 3.7 years (control group A) and eight children with neutropenia and solid tumors but not treated with rHuGM-CSF, aged 7.2 ± 4.0 years (control group B), were included in the study as control groups. The solid tumors of control group B were osteosarcoma (3 cases), neuroblastoma (4 cases) and Askin's tumor (1 case). The neutrophil response to rHuGM-CSF and the duration of the neutropenic phase in children with solid tumors were compared with control group B. Endogenous serum GM-CSF, IL-3, and IL-4 levels were measured in patients before and after rHuGM-CSF therapy and in control group A.

The peripheral blood samples for analyzing plasma GM-CSF, IL-3, and IL-4 concentrations were collected one day before and after rHuGM-CSF treatment. The plasma GM-CSF (Amersham, UK), IL-3 (Amersham), and IL-4 (Amersham) levels were analyzed using enzyme-linked immunosorbent assay (ELISA). The samples were stored at -30 °C until use. The minumum detectable concentrations of plasma GM-CSF, IL-3 and IL-4 by this method were 0.3 pg/ml, 1.5 pg/ml and 0.6 pg/ml, respectively.

Hematological parameters including white blood cell (WBC), absolute neutrophil count (ANC) and platelet count were investigated with a hemocounter (Serono Baker System 9000, Allentown, PA, USA) and through peripheral blood smear analysis. Liver and renal function tests were performed in order to determine toxic side effects. The patients were also observed for clinical side effects such as fever.

The differences and correlations between different cytokines and between cytokines and hematological parameters were analyzed using Mann-Whitney U test and regression analysis.

Results

Results related to neutrophil response and side effects after rHuGM-CSF treatment:

Before rHuGM-CSF therapy, ANCs of two patients were above 1000/mm^3 (patient nos, 2 and 5). Thus, prompt neutrophil recovery occurred in seven of eight children (Table II). Patient no. 7 did not show any increase in ANC because of the severe myelosuppression. The ANC rose above 1000/mm^3 after three days (median) of the rHuGM-CSF treatment (range 1-5 days) (Table II). However, the median time for ANC to rise above 1000/mm^3 in control group B was seven days (range 5-12 days).

These results showed that rHuGM-CSF treatment significantly decreased the duration of the neutropenic phase in children with solid tumors ($p < 0.001$).

Both groups of patients with solid tumors (treated with or without rHuGM-CSF) had no clinical signs of severe infection during the follow-up periods. Clinical and laboratory side effects of the rHuGM-CSF treatment are listed in Table II.

Results related to endogeneous plasma GM-CSF, IL-3 and IL-4 concentrations in study group and in healthy controls: The median values and ranges of endogenous plasma GM-CSF, IL-3, and IL-4 concentrations in rHuGM-CSF treated patients with solid tumors and in healthy controls are listed in Table III.

Table III: Median Plasma Levels of GM-CSF, IL-3 and IL-4 in the Study Group and in Healthy Children (pg/ml)

	GM-CSF	IL-3	IL-4
Before rHuGM-CSF	3.1 (< 0.3-455)	< 1.5 (< 1.5-50)	5.1 (0.6-41.5)
After rHuGM-CSF	15.6 (3.1-28.1)	< 1.5 (< 1.5-15.6)	0.6 (0.6-20.8)
Healthy controls	< 2.6 (< 0.3-7.3)	< 1.5 (< 1.5-1.5)	< 0.6 (< 0.6)
p value	1-2: $p < 0.001$ 1-3: $p < 0.05$ 2-3: $p > 0.05$	1-2: $p > 0.05$ 1-3: $p < 0.05$ 2-3: $p > 0.05$	1-2: $p < 0.001$ 1-3: $p < 0.05$ 2-3: $p > 0.05$

No significant correlation has been found ($p > 0.05$) between plasma GM-CSF concentration and ANC or platelet count in the peripheral blood of the study group. However, IL-3 levels of this neutropenic group positively correlated with the platelet count (r: 0.680, $p < 0.01$), but not with the ANC.

Plasma IL-4 concentrations, however, were positively correlated with the level of GM-CSF in the same individual (r: 0.87, $p < 0.001$).

The endogeneous plasma GM-CSF and cytokine levels before rHuGM-CSF treatment were compared to those in control group A (healthy controls). Not only the plasma GM-CSF level, but also IL-3 and IL-4 levels in the neutropenic solid tumor group were found to be significantly higher than those in healthy children ($p < 0.05$).

The median plasma GM-CSF level increased from 3.1 pg/ml to 15.6 pg/ml after rHuGM-CSF treatment (Table III), and there was a significant difference ($p < 0.01$) in plasma GM-CSF levels obtained before and after treatment. In contrast, plasma IL-4 values were found to be significantly lower ($p < 0.001$) than initial values after the administration of GM-CSF exogenously.

In addition, cytokine levels of the patients with osteosarcomas (n: 5) and patients with the other tumours (n: 5) were compared in the study group and plasma IL-4 levels were found to be significantly elevated in the osteosarcoma group ($p < 0.05$).

Discussion

CSFs both G-CSF and GM-CSF, are supportive care measures designed to reduce the likelihood of neutropenic infections that result from chemotherapy[13, 14]. Recently, investigators have started to use rHuGM-CSF or rHuG-CSF in pediatric solid tumors in order to accelerate neutrophil recovery. Weinthal et al.[15] reported that exogenous G-CSF therapy when used as an adjunct to aggressive chemotherapy in children with solid tumors reduced both the duration of neutropenia and of infection. On the other hand, Fink et al.[3] treated septic neutropenic pediatric cancer patients with rHuGM-CSF, and an ANC above 500/mm^3 was reached after five days (median). In our study, median time for the ANC to rise above 1000/mm^3 after rHuGM-CSF therapy was three days. When compared to the other pediatric solid tumor patients not treated with rHuGM-CSF, this therapy significantly reduced the duration of the neutropenic phase.

rHuGM-CSF reduces the frequency of infections, antibiotic usage and the duration of hospitalization because it enhances not only the number and functions of neutrophils but also the cytotoxic functions of monocytes and macrophages[1, 16, 17]. None of the patients in the study or in the control groups had any infections. As a result, no significant differences were observed with regard to duration of antibiotic usage and hospitalization and no conclusion could be made about the reduction of the frequency of infections.

rHuGM-CF was well tolerated were no documented cases of bone pain or of local irritation at the site of injection. rHuGM-CSF-induced fever was observed in three cases (30%). Significant differences in platelet counts have been reported in 1-12 percent of patients with various diseases under rHuGM-CSF treatment. In our study, thrombocytopenia was observed in 20 percent of the patients after rHuGM-CSF therapy. There was no indication of inadvertent side effects from serum levels of bilirubin, aspartate-aminotransferase (AST), protein, creatinine, or from urine analyses.

Despite the growing clinical use of the CSFs, there is limited information available about the pattern of endogenous cytokine responses in patients with solid tumors. It has been reported that after intensive chemotherapy (CT) and/or radiotherapy (RT), GM-CSF was released into the serum[18]. Galdiero et al.[19] found an increased release of IL-4 from lymphocytes after minimum doses of irradiation. In our study, before rHuGM-CSF therapy, the endogenous plasma GM-CSF, IL-3, and IL-4 levels in patients who had solid tumors and were treated with CT/RT were found to be significantly higher than those in healthy controls. In patient no. 7, who had both chemotherapy and radiotherapy, the plasma GM-CSF level was extremely high (455.0 pg/ml). The increase in GM-CSF, IL-3, and IL-4 levels may be due to CT/RT. On the other hand, IL-4, G-CSF, IL-1, IL-5, IL-6, and tumor necrosis factor alpha (TNF-α) have been shown to be secreted by malignant cells in Hodgkin's disease[12]. Statistically significantly higher levels of IL-2, soluble IL-2 receptor, IL-6, IL-8, IL-10, and TNF-alpha were observed in patients with non-Hodgkin's lymphoma and acute lymphoblastic leukemia as compared to controls[20,21]. It has been demonstrated that osteosarcoma cell lines were potent producers of IL-11 which is an important component of the cytokine network[22]. With regard to these findings, the increase in plasma GM-CSF, IL-3 and IL-4 levels in pediatric patients with solid tumors, including osteosarcomas, may be due to the secretion of GM-CSF and these cytokines by tumor cells. Furthermore, IL-4 has an anti-tumor activity[2,6,8]. IL-4 and GM-CSF as well as TNF and IL-2 have been reported to activate macrophages for the destruction of tumor cells[23]. The increased production of GM-CSF and IL-4 by lymphocytes and macrophages in patients with solid tumors may be a part of host defense against malignancy.

IL-4 modulates the activity of a variety of lymphoid, hemopoietic and mesenchymal cells, but little is known about its influence on bone cells[24]. In our study, IL-4 levels of the patients with osteosarcomas were significantly higher than those of patients with other tumors. Elevated IL-4 levels in our patients with osteosarcomas may have exerted a modulatory effect on osteosarcoma cells, as was described in Riancho et al.'s[24] study.

Omori et al.[4] and Cebon et al.[5] reported that the peripheral neutrophil count correlates with the serum G-CSF level, but not with GM-CSF. The results of our study supported the data of these studies, because no significant correlation has

been found between plasma GM-CSF level and ANC and platelet count in the peripheral blood of the patients. On the other hand, IL-3 is a multi-colony stimulatory factor and is involved in proliferation of pluripotent stem cells to produce all types of hemopoietic cells, including megakaryocytes and platelets[25]. In our study, plasma IL-3 levels were found to be positively correlated with the platelet count.

GM-CSF is secreted by T lymphocytes, macrophages, fibroblasts, endothelial cells and dendritic cells, whereas IL-4 is produced by activated T helper cells[25,26]. T helper cells are the sources of both GM-CSF and IL-4. Some of the cytokines that control hematopoiesis are present at all times (G-CSF, erythropoietin) whereas others are produced in response to specific stimuli (IL-3, IL-4, GM-CSF)[27]. In this study, plasma IL-4 concentrations were positively correlated with the GM-CSF level in the same individual, suggesting that they are produced in response to the same stimuli.

rHuGM-CSF has been known to be eliminated from the serum with in two or three hours after its subcutaneous administration, and serum levels decrease to undetectable values after 24 hours[17]. Therefore, theoretically, rHuGM-CSF treatment does not increase endogenous plasma GM-CSF levels one day after its administration. However, there was a significant difference in plasma GM-CSF levels obtained before and after rHuGM-CSF treatment. Because of its pharmacokinetic properties, this elevation was not thought to be related to rHuGM-CSF therapy. The half-life for E.coli derived GM-CSF administered subcutaneously in a dose of 5.5 μg/kg had been reported to be 2.1 hours by Nissen and Hovgaard[28].

Plasma IL-4 values were found to be significantly lower than intial values after administration of rHuGM-CSF. There is only one other study in which cytokine levels were measured before and after chemotherapy and rHuGM-CSF treatment. In this study, cytokine levels progressively declined to normal ranges in responding patients[20]. The decline in plasma IL-4 values in our study was similar with Stasi et al.'s[20]. data.

In conclusion, rHuGM-CSF treatment significantly decreased the duration of the neutropenic phase in children with solid tumors. There is no correlation between endogenous plasma GM-CSF concentrations and absolute neutrophil count, but IL-3 concentrations were found to be correlated with platelet counts. The endogenous plasma GM-CSF, IL-3, and IL-4 levels were significantly higher in pediatric patients with solid tumors. The increased productions might be related to their CT-RT regimen or to secretion by malignant cells. These results demonstrate that cytokine measurements can provide valuable information for better management of pediatric solid tumors. In the future, they may be useful in monitoring disease activity or may be used as prognostic indicators.

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