The effect of recombinant GM-CSF on IL-6 and TNF-α levels in epithelial ovarian cancer patients who received paclitaxel and cisplatinum: preliminary results

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Summary

Objective: We investigated the effects of GM-CSF factor on IL-6 and TNF-α levels prior to paclitaxel-cisplatinum combination chemotherapy for advanced epithelial ovarian cancer.

Materials and Methods: Twenty-three consecutive patients with FIGO (International Federation of Gynecology and Obstetrics) Stage III-IV epithelial ovarian cancer were enrolled in the study. Following cytoreductive surgery patients received 175 mg/m^2 paclitaxel and 75 mg/m^2 cisplatinum on the same day. These 23 patients also received RhuGM-CSF five days before at a dose of 5 μg/kg/day by subcutaneous injection for three days. IL-6 and TNF-α levels were measured before and 24 hours later following the last dose of RhuGM-CSF.

Results: White blood cell counts on the 10th day of the cycle were lower than preGM-CSF white blood cell counts and the difference was statistically significant (p = 0.003). Platelet levels on the 10th day of the chemotherapy cycle were lower than pre GM-CSF levels, however were not statistically significant (p = 0.097). Post GM-CSF TNF-α and IL-6 levels were higher than pre GM-CSF levels. This difference was statistically significant for TNF-α (p = 0.002) however for IL-6 a statistically significant difference was not detected (p = 0.55). GM-CSF does not significantly effect IL-6 levels in contrast to TNF-α.

Conclusion: Clinical implications of increased levels of TNF-α are unclear and for a precise determination further studies are needed.

Key words: Recombinant GM-CSF; IL-6; TNF-α; Epithelial ovarian cancer.

Introduction

Cytokines are intracelluler hormones believed to play a functional role in the natural history of malignant disease such as a stimulatory role by modification of cytokine expression on neoplastic cells or an inhibitory effect by generating host anti-tumor response [1]. Cytokines are integral to the function of the immune system. However they have profound effects on virtually every organ system such as bone remodelling and regulation of hematopoiesis [2]. Interleukin 6 (IL-6) has a stimulatory effect on hematopoiesis and is a key regulator of inflamatory response to several antigens. However a poor prognosis in patients with multiple myeloma, renal cell carcinoma and ovarian cancer has been associated with elevated IL-6 levels [3]. In ovarian cancer elevated IL-6 levels have been associated with poor prognosis and IL-6 activity was higher in serous and mucinous than endometrioid and undiffentiated ovarian cancer [4, 5] Tumor necrosis factor alpha (TNF-α) is another well known mediator of inflamatory processes as well as having detrimental effects on the metabolism of patients with neoplastic disorders such as cancer-related fatigue [6].

Recently, higher 5-year survival rates and better responses have been produced in epithelial ovarian cancer by paclitaxel and platinum-based chemotherapy. However, myelosupression is still the major limiting factor [7]. GM-CSF is a member of the cytokine family that stimulates proliferation, differentiation and activation of undifferentiated myeloid progenitor cells to mature granulocytes and macrophages. Several cytokines could be used to accelerate recovery of hematopoiesis after chemotherapy. GM-CSF is one of them and the therapeutic effects of GM-CSF such as reduction of the neutropenic and thrombocytopenic period and accelerated proliferation of myeloid cell lines have been seen in breast and lung cancer, acute leukemia, multiple myeloma, lymphoma and other solid tumors [8]. In this study we investigated the effects of GM-CSF factor on IL-6 and TNF-α levels prior to paclitaxel-cisplatinum combination chemotherpy for advanced epithelial ovarian cancer.

Materials and Methods

Twenty-three consecutive patients with FIGO Stage III-IV epithelial ovarian cancer were enrolled in this study. Patients who had severe heart, lung, liver or kidney dysfunction were excluded. Following cytoreductive surgery patients received 175 mg/m^2 paclitaxel (Taxol, Bristol-Myers Squibb) and 75 mg/m^2 cisplatinum (Cisplatin DBL, Orna) on the same day (day 1). Dexamethason (Dekort, Deva Co, Istanbul), 16 mg, was administered intravenously as a part of the prophylaxis for

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hypersensitivity. In addition forced diuresis with 20 mg IV Furosemid (Desal, BioFarma Co, Istanbul) was performed with synchoronous isotonic fluid load. These 23 patients also received RhuGM-CSF (Molgramostim, Leucomax, Novartis) five days before at a dose of 5 μg/kg/day by subcutaneous injection for three days. IL-6 and TNF-α levels were measured before and 24 hours after the last dose of RhuGM-CSF. White blood cell count and platelet count were measured before and after RhuGM-CSF administration on the tenth day of the cycle.

Venous blood (10 ml) was drawn in the serum separating tubes. Serum samples were centrifugated at 3,000 rpm for 15 minutes and the serum frozen and stored at -20°C. IL-6 and TNF-α were assayed using chemiluminescence (DPC-immulite). The limits of detection of IL-6 and TNF-α assays were 5 pg/ml and 4 pg/ml, respectively.

Statistical analysis

Data were not normally distributed and therefore comparison between the values before the Wilcoxon rank-sum test, with significance defined as $p < 0.05$, was performed before and following the RhuGM-CSF. Median values were reported with standard deviations and ranges, respectively.

Results

Between 1998 and 2001, 23 patients were enrolled in the study. Fourteen had FIGO Stage III epithelial ovarian cancer (60.8%) and nine had Stage IV disease. The median age was 50 years old. Other demographic and clinical features are summarized in Table 1. White blood cell counts on the 10th day of the cycle were lower than preGM-CSF white blood cell counts and this difference was statistically significant ($p = 0.003$). Platelet levels on the 10th day of the chemotherapy cycle were lower than preGM-CSF levels however was not statistically significant ($p = 0.097$). PostGM-CSF TNF-α and IL-6 levels were higher than preGM-CSF levels. This difference was statistically significant for TNF-α ($p = 0.002$) however for IL-6 statistically significant difference was not detected ($p = 0.55$) (Table 2).

Table 1. — *Demographic and clinical features of 23 patients who received RhuGM-CSF.*

	Median ± Standard deviation
Age	50 ± 10.3177 (25-67)
Gravida	3,3478 ± 2.9015 (0-10)
Parity	2.5217 ± 1.9275 (0-6)
Body Mass Index (kg/m²)	21.5891 ± 0.69 (21.45-22.70)
Stage	
FIGO Stage III	14
FIGO Stage IV	9
Histopathologic diagnosis	
Serous adenocarcinoma	15
Mucinous adenocarcinoma	3
Endometrioid adenocarcinoma	1
Undifferentiated carcinoma	2
Clear cell carcinoma	2
WHO Performance Score	
0	0
1	19
2	4

Table 2. — *Comparison of white blood cells, platelet counts and IL-6, TNF-α levels before and after RhuGM-CSF administration.*

	Pre GM-CSF	Post GM-CSF	p value*
White Blood Cell Count (/mm³)	6671 ± 1547.93 (3200-10200)	4288.69 ± 825.184 (3180-6050)	p = 0.003
Platelet Levels (/mm³)	283652 ± 88573.85 (168000-482000)	257521.73 ± 66424.16 (167000-425000)	p = 0.097
IL-6 Levels (pg/ml)	20.41 ± 19.10 (5.00-60.60)	22.90±17.01 (5.40-58.10)	p = 0.55
TNF-α Levels (pg/ml)	15.07 ± 14.71 (4.00-46.80)	20.62 ± 14.83 (4.90-48.70)	p = 0.002

*$p < 0.05$ = statistically significant.

Discussion

Epithelial ovarian cancers are responsible for major gynecologic cancer-related mortality in Western countries. Recently, higher 5-year survival rates and better responses have been produced in epithelial ovarian cancer by paclitaxel and platinum-based chemotherapy. However chemotherapy-related bone marrow toxicity is the major limiting side-effect of antineoplastic drug therapy [7]. Cycle canceling due to neutropenic fever, thrombocytopenia, bleeding and anemia has a major impact on the patient's quality of life and can cause fatal complications. Bone marrow supression reaches a peak level between the 7th day and 10th day following chemotherapy administration. Therefore recently hematopoietic growth factors have been used to overcome these major complications. Granulocyte macrophage-colony stimulating factor (GM-CSF) is a member of the cytokine family that stimulates proliferation, differentiation and activation of undifferentiated myeloid progenitor cells to mature granulocytes and macrophages. Also GM-CSF enhances expression of adhesion molecules (integrin family), antifungal activity of monocytes, priming for the synthesis of 5-lipoxygenase products, production of superoxide anion and platelet activating factor (PAF) by neutrophils [9-11]. GM-CSF had synergistic or additive effects with IL-1, IL-3, IL-6, IL-11 and erythropoietin [12, 13]. RhuGM-CSF therapy can improve a patient's quality of life by reducing the morbidity associated with myelosupression. However the stimulatory effect of RhuGM-CSF on IL-6 and TNF-α levels is unknown as is whether it causes progression of disease or not.

Patients with ovarian cancer have elevated levels of IL-6, TNF-α in serum and ascitic fluid [14]. Elevated concentrations of IL-6 correlated with poor final outcome, and increased IL-6 and IL-8 levels correlated with poor initial response to chemotherapy. Treatment with paclitaxel is associated with an increase in expression of a limited number of cytokines in patients with ovarian cancer notably IL-6, IL-8 and macrophage chemotactic protein (MCP) [15]. Tempfer *et al.* [16] found that IL-6 levels were correlated with FIGO stage, however lymph node involvement and grade were not correlated with IL-6 levels. Plante *et al.* [17] reported that IL-6 levels in ascites fluid were higher than serum levels. Also a corre-

lation between the IL-6 levels and ascites volume was reported by the same authors. In this study however postGM-CSF IL-6 and TNF-α levels were higher than preGM-CSF levels however for IL-6 this difference was not statistically significant ($p > 0.05$).

Due to a short follow-up period the relationship between the postGM-CSF IL-6 levels and survival is not reported. Moreover these results were yielded from only one chemotherapy cycle. GM-CSF did not significantly effect IL-6 levels in contrast to TNF-α. Therefore administration of RhuGM-CSF could not effect the progression of disease in patients. However the clinical implication of an increased level of TNF-α is unclear and for precise indications further studies are needed.

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