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ORIGINAL ARTICLE

Effects on the immune system and toxicity of carboplatin/paclitaxel combination chemotherapy in patients with stage III-IV ovarian and non small cell lung cancer and its role in survival and toxicity

K. Hatziveis[1], D. Tourlakis[2], P. Hountis[3], I. Kyriazanos[4], M. Sougleri[5], P. Ginopoulos[5], C. Camoutsis[1]

[1]Laboratory of Pharmaceutical Chemistry, Department of Pharmacy, Faculty of Pharmacy, School of Health Sciences, University of Patras, Patras; [2]Department of Obstetrics and Gynecology, General Hospital "St Andreas", Patras; [3]Department of Thoracic Surgery, Naval Hospital of Athens, Athens; [4]Department of General Surgery, Naval Hospital of Athens, Athens; [5]Department of Clinical Oncology, Chemotherapy Unit, General Hospital "St Andreas", Patras, Greece

Summary

Purpose: *To examine the impact of paclitaxel and carboplatin combination chemotherapy on the parameters of the immune system in patients with non small cell lung cancer (NSCLC) and with ovarian cancer before, during and after chemotherapy, and the effect of this combination on the overall patient survival.*

Methods: *24 patients with NSCLC and 20 with ovarian cancer (all in stage IIIb-IV) treated with 6 courses of paclitaxel and carboplatin combination chemotherapy were separated into two groups according to their survival group A: long survival (> 12 months for NSCLC; > 30 months for ovarian cancer) group B: short survival (<12 months for NSCLC; <30 months for ovarian cancer). At the same time we studied some immunological parameters (CD3, CD4, CD8, CD56, CD34, IL-3, IFN-γ) in relation with the induced toxicity during chemotherapy. The results were analysed using the ANOVA method.*

Results: *We observed a statistically significant difference of CD4 and CD4/CD8 after chemotherapy between groups A and B ($p<0.001$ and $p< 0.006$ respectively), implying that the further increase of T-helper cells after chemotherapy had a positive impact on survival. In addition, statistically interesting was the difference in values of IFN-γ between patients of groups A and B before and after chemotherapy ($p< 0.039$ and $p< 0.027$, respectively). Patients with high IL-3 had little chance of toxicity.*

Conclusion: *Our findings support that with carboplatin/paclitaxel combination chemotherapy, important parameters of the immune system (IFN-γ, CD4, CD4/CD8) can be used as prognostic factors for survival, while others (IL-3) as indicators of toxicity.*

Key words: carboplatin, immune system, paclitaxel, survival, toxicity

Introduction

Traditionally, the goal of chemotherapy has been seen as direct cytotoxicity and induction of tumor cell death. However, the immunoadjuvant effect of chemotherapy, where the immune response induced by tumor cell death mediates the suppression of tumor growth and determines the long-term survival of patients, is now well established [1].

It is known from several studies that sometimes small doses of chemotherapy can cause activation of defense mechanisms, such as cellular immunization and antibody production by direct effects on lymphocytes and their subpopulations. Then the production of cytokines is triggered by the above procedure [2-6].

A large number of chemotherapeutic agents causes immunosuppression (Table 1) [7]. Taxanes are potent chemotherapeutic agents with significant efficacy in various malignancies such as those of the breast, ovaries, lung and other organs [8].

Paclitaxel, which was originally extracted from the bark of the Pacific yew tree Taxus brevifolia, is the first and most representative of the pharmaceutical taxane family [9]. Its tumoricidal action is based on the

Correspondence to: Konstantinos Hatziveis, MD. Aristomenous 65, 241 00 Kalamata, Greece. Tel / fax: +30 27210 94155, E-mail: khatziveis@gmail.com
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Table 1. Immunosuppressive agents

High immunosuppression	*Low immunosuppression*
Cyclophosphamide	Busulfan
Nitrosoureas	Dacarbazine
Purine analogues	Bleomycin
Pyrimidine analogues	Doxorubicin
Epirubicin	Mithramycin
Folic acid antagonists	
Vinca alkaloids	
Antibiotics	
Corticosteroids	

ability to prevent the de-polymerization of the cytoskeleton's microtubules in free tubulin, ultimately inhibiting the proliferation of tumor cells [10].

However, paclitaxel also affects the immune system causing antineoplastic activity. It regulates cytokine production, activates or inhibits lymphocytes that exert inhibitory action on various tumors and activates macrophages, leading them subsequently to chemotaxis of cytotoxic lymphocytes or to cytotoxic cytokine production [11].

The purpose of this study was to investigate the impact and the outcome of a combination of carboplatin and paclitaxel on the immune system before and after chemotherapy, as well as the impact of this combination on the survival of patients with NSCLC and ovarian cancer. In addition, we combined the findings with the induced toxicity during chemotherapy.

Methods

The study included 44 patients who were admitted in the Chemotherapy Unit of the General Hospital of Patras "Aghios Andreas", from January 2005 to February 2008. Twenty-four of them had NSCLC (stage IIIb-IV) and 20 ovarian cancer (stage IIIb-IV). Average age was 67±7.8 years. The patients were separated into 2 groups in terms of survival as follows:
Group A (n=15): patients with long survival (> 12 months for NSCLC and > 30 months for ovarian cancer).
Group B (n=29): patients with short survival (<12 months for NSCLC and <30 months for ovarian cancer).

We also divided all patients into 2 groups according to the levels of IL-3 found before and during chemotherapy:
Group C (n=20) IL-3: 124-265 (high levels) group D (n=24) IL-3: 43-110 (low levels)

Chemotherapy

Chemotherapy consisted of i.v. paclitaxel 200 mg/m^2 and carboplatin 300 mg/m^2, both on day 1 and repeated every 3 weeks.

Lymphocyte subgroups and cytokines determinations

Three venous blood samples of 10 ml each were collected from each patient for subsequent differentiation of the lymphocyte subgroups. The first sample was collected one week before starting therapy, the second one week before the start of the 3rd chemotherapy cycle and the third one month after the end of the 6th cycle of chemotherapy.

All samples were analysed for determination of both the absolute number and the percent of total lymphocytes and their subgroups: Total number of T cells (CD3), T-helper (CD4), T-suppressors (CD8), natural killer (NK) cells (CD56) and precursor stem cells (CD34). Aliquots of 100 μl of whole blood, anticoagulated with EDTA, were incubated with appropriate combinations of fluorescence-conjugated monoclonal antibodies. After lysing of erythrocytes (FACS lysing solution, BD Biosciences, Heidelberg, Germany), cells were washed once with PBS, 1% FCS, and 0.2% NaN3 (Biocult Laboratories, Glasgow, UK) before measurement.

The monoclonal antibodies used were: anti-Leu 1 for CD3, anti-Leu 3a for CD4, anti-Leu 2a for CD8, anti-Leu 11b for CD56 and for CD34 (Becton-Dickinson, San Jose, CA, USA). Characterization of the lymphocytic phenotypes were made using immunofluorescence microscope (B-353LD1 OPTIKA, Italy) at ×400 magnification.

Furthermore, calculation of the ratio of T-helper/T-suppressor cells (CD4/CD8), and calculation of cytokines (IL-3 and IFN-γ) as a result of stimulation of peripheral mononuclear hematopoietic cells using endotoxin (LPS-lipopolysaccharide) and phytohaemagglutinin (PHA) after 24h lymphocyte cultures were performed.

The composition of cytokines was determined by the ELISA method with the use of an enzyme-linked immunosorbent kit (Medgenix, Medgenix Diagnostics, Belgium). The positivity threshold was 10 pg/ml for IL-3 and 5 pg/ml for IFN-γ.

Statistical analysis

Statistical analysis was carried out using the ANOVA method. The rates were expressed as standard error of the mean (SEM) and, in case of non-standard distribution, as median, while categorical variables were described as percentages.

The comparison of the groups in relation to the levels of individual variables, e.g. lymphocyte subpopulations, IFN-γ, IL-3 etc, was conducted using the one-way ANOVA. The model analysis that was used was ANOVA type II SS (sums of squares) with 95% confidence interval. All tests were two-sided. Statistically significant were considered values of $p<0.05$ and highly significant values of $p<0.01$. All the statistical analyses were performed with the SPSS statistical package, version 16.00 (SPSS Inc, Chicago, IL).

Results

Tables 2 and 3 show the impact of the tested chemotherapeutic regimen on the total T-cell number and their subpopulations at each phase of the study, in relation to either long or short survival.

It was remarkable that the absolute number of T lymphocyte subpopulations showed a marked tendency to decrease during chemotherapy and subsequently to increase more than the initial values.

This finding was particularly evident in patients with long survival (group A) but without statistical significance in most of the measurements. The same results were observed regarding the two cytokines examined (Table 4). Concerning toxicity the following results were taken:

Table 2. Percents of T-lymphocyte subpopulations (mean±SD), before, during and after chemotherapy. A: long survival group, B: short survival group

T-lymphocytes	*Patient group*	*Before chemotherapy (1)*	*During chemotherapy (2)*	*After chemotherapy (3)*
CD3	A	47.25±4.92	50.12±2.81	53.62±3.38
	B	43.87±2.37	47.00±4.86	47.12±2.54
CD4	A	30.75±3.75	30.62±2.72	41.50±4.58
	B	22.75±4.04	27.62±4.34	20.00±1.91
CD8	A	25.00±3.71	24.25±2.15	25.37±2.67
	B	18.87±2.34	19.00±2.07	24.25±3.06
CD4 / CD8	A	1.30±0.14	1.46±0.17	1.66±0.16
	B	1.35±0.30	1.49±0.22	0.93±0.15
CD56	A	12.50±2.6	17.66±2.87	16.83±4.09
	B	11.50±1.69	12.00±2.61	22.25±1.43
CD34	A	5.00±1.22	2.50±0.59	8.87±3.42
	B	4.12±0.81	2.87±0.74	4.87±1.86

CD3= total T cells, CD4= T-helpers, CD8= T-suppressors, CD56= NK cells, CD34= haematopoietic precursor cells; Statistical significance: CD4: 3A-3B: p=0.001, CD4 /CD8: 3A-3B: p=0.006, CD34: 1A-2A: p=0.03 and 2A-3A: p=0.048

Table 3. Absolute number of T-lymphocyte subpopulations (mean±SD) before, during and after chemotherapy. A: long survival group, B: short survival group

T-lymphocytes	*Patient group*	*Before chemotherapy (1)*	*During chemotherapy (2)*	*After chemotherapy (3)*
CD3	A	664. 96±104.39	535.97±104.97	744.13±137.97
	B	498.54±115.11	388.71±98.54	638.08±259.32
CD4	A	516.55±104.41	324.95±45.39	516.80±94.29
	B	286.54±67.99	212.24±62.65	287.38±104.09
CD8	A	359.75±29.31	281.20±32.52	484.56±95.84
	B	186.59±52.13	143.38±36.69	287.87±116.12
CD56	A	177.96±49.29	122.58±19.49	262.77±91.95
	B	174.32±48.69	107.85±31.01	308.56±155.54
CD34	A	64.22±17.03	20.23±6.39	125.75±48.33
	B	45.12±17.39	21.24±8.64	55.49±22.10

CD3= total T cells, CD4= T-helpers, CD8= T-suppressors, CD56= NK cells, CD34= haematopoietic precursor cells; Statistical significance: CD8: 1 A-1 B: p= 0.012, and 2 A-2 B: p=0.014; CD34: 1 A-3 A: p=0.003, and 2 A-3 A: p=0.04

Concerning the patients in group C, G-CSF administration was not necessary in 5 patients (25%) during chemotherapy, while it was necessary in 15 patients (75%) only after the 5th or the 6th cycle of chemotherapy. In the same group only 3 patients received antibiotics *per os*, while 4 of them needed erythropoietin administration for grade 2-3 anemia.

Conversely, in group D, G-CSF administration

Table 4. Cytokine synthesis (pg / ml; mean ± SD) by phytohaemagluttinin (PHA) or endotoxin (lipopolysaccharide-LPS) stimulated peripheral blood mononuclear cells. A: long survival group B: short survival group

Cytokines	*Patient group*	*Before chemotherapy (1)*	*1 week before the 3rd cycle (2)*	*1 month after the last 6th cycle (3)*
IL-3				
by PHA	A	100.25±19.45	77.75±12.79	166.75±32.75
	B	190.87±41.32	115.37±33.21	236.62±45.80
by LPS	A	147.67±47.16	86.50±27.69	282.62±80.32
	B	221.50±51.22	98.00±25.52	358.75±115.51
IFN-γ				
by PHA	A	483.87±139.06	310.00±71.49	607.00±156.63
	B	160.50±28.57	162.12±64.37	207.00±38.87
by LPS	A	454.75±69.75	263.37±48.21	691.50±63.57
	B	280.50±61.09	156.37±44.90	313.75±56.11

IL-3= interleukin-3, IFN-γ= interferon-γ
Statistical significance: IL-3 (by PHA): 2 A-3 A: p=0.024, and 2 B-3 B: p=0.048; (by LPS): 2 A-3 A: p=0.024, and 2 B-3 B: p=0.049; IFN-γ (by PHA): 1 A-1 B: p=0.039, and 3 A-3 B: p=0.027; (by LPS): 1 A-2 B: p=0.042, 1 A-3A: p=0.025, 2 A-3 A: p < 0.001, 2 B-3 B: p=0.047, 3 A-3 B: p=0.001

Table 5. Levels of IL-3 between patients with low toxicity (group C) and high toxicity (group D) before and during chemotherapy and its impact on the induced toxicity

IL-3	*Patient group*	*Before chemotherapy (1)*	*During chemotherapy (2)*
by PHA	C	220.37±30.8	132.50±86.36
	D	70.00±8.63	60.62±23.00
By LPS	C	267.50±52.25	125.5±28.61
	D	101.87±23.34	49.12±9.06

Statistical significance: IL-3 (by PHA): 1C-1D: p=0.002, and 2C-2D: p=0.039; IL-3 (by LPS): 1C-1D: p=0.012, and 2C-2D: p=0.012

was required in 22 patients (92.08%) after the 1st and 2nd cycle of chemotherapy. From this particular group 8 patients (33.3%) experienced grade 3-4 febrile neutropenia. Fourteen patients (58.3%) had grade 2-3 anemia and were given erythropoietin while in 4 of them blood transfusion was necessary. Six patients (25%) developed pulmonary infection and 4 of them (16.6%) mild diarrhea.

Peripheral neuropathy occurred in 5 (25%) patients from group C and 6 (25%) from group D.

Altogether, 20% of the patients experienced significant toxicity in group C (with high levels of IL-3) and 80% in group D (with low levels of IL-3).

Table 5 shows the levels of IL-3 before and during chemotherapy between groups C and D. We observed that the production of IL-3 by PHA, between the two groups was statistically significant both before (p=0.002) and during chemotherapy (p=0.0039). Furthermore, the production of IL-3 by LPS both before and during chemotherapy was also statistically significant (p=0.012).

Discussion

According to the general opinion that the development of cancer reflects mostly a malfunction of the immune system, we examined the prognostic significance of some immune parameters in relation to long or short survival in patients with NSCLC and ovarian cancer treated with paclitaxel plus carboplatin combination chemotherapy. Chemotherapy is the most effective therapy in the treatment of metastatic solid tumors. Various chemotherapeutic agents are currently used either as mono-or a multi-agent treatment, thereby affecting the human immune system in many ways. Sometimes, after using conventional chemotherapy, an unexpected increase in the immune system activity is observed, suggesting that many agents (actinomycin D, doxorubicin, dacarbazine, methotrexate) alter the cellular membrane of cancer cells and subsequently the reaction of the immune system [12].

In this study we investigated the effects of the combination of carboplatin and paclitaxel on the immune system. A similar study has also been reported in the recent past by Geller et al. [13] and Koshiba et al. one year ago [14]. Our study also showed a statistically significant difference between groups A and B after chemotherapy, in terms of the CD4 subpopulation (A / B, p=0.001) and the ratio CD4 / CD8 (A / B, p=0.006). This shows that the further increase of T-helper cells reflects the improved prognosis for patient survival. A recent study by Javeed et al. ended with the same conclusion for the effect of paclitaxel on the immune cells [15]. As for the CD34 cells, a significant difference came forward between patients of group A, indicating that after the initial repression during chemotherapy, there was a further sensitization and increase in their number after the end of treatment. Observing the same cells, and in particular their absolute number, it was proved that they followed the same pattern after 3 measurements between patients of group A. A big drop during chemotherapy was followed by a sharp increase at the end of it, so that the absolute cell number was higher than its initial value.

Concerning the CD8 cells, the present study showed low absolute numbers both before and during chemotherapy, obviously related to the short survival among group B patients. On the other hand, it is noteworthy that in group A patients the absolute numbers of these cells remained high during chemotherapy and characteristically they increased after it, without though showing statistical significance during measurements. At this point, Stumpf et al. in their study concluded that the intraepithelial infiltration of ovarian carcinomas with CD8-positive T lymphocytes was prognostic for improved survival in optimally debulked, stage III ovarian cancer patients, most significantly also for those with the option of adjuvant paclitaxel /carboplatin chemotherapy [16].

Observing the values of IL-3 we noticed that there was a statistically significant difference, both during and after chemotherapy, in both groups (A and B), and both after stimulation with PHA and LPS. Although the production of IL-3 appears not to have particular relation to the survival and cannot be considered as prognostic factor, it seems, however, related to induced toxicity in patients with cancer. Summarizing, it can be said that the levels of IL-3 in patients with cancer, both

before and during chemotherapy, can be a useful prognostic indicator of the likelihood of high or low toxicity.

While levels of IL-3 before the start of treatment are a prognostic indicator of potential toxicity, during chemotherapy they determine, along with other factors (stage or cancer, physical condition of patients) where the use of chemotherapy can cause further complications (neutropenia, anemia, infections etc) and the same or increased toxicity.

Unlike that, observing the IFN-γ after stimulation with PHA, we noticed high levels before and during treatment in group A patients compared with patients of group B ($p=0.039$ vs. $p=0.027$). These levels prove that this cytokine is considered an important prognostic factor for the survival of the patients in our study.

Similarly, the levels of IFN-γ after stimulation with LPS in group A patients revealed a statistically significant value compared with patients in group B (p (A) <0.001 vs. p (B)$=0.001$). IFN-γ plays a stimulatory role for macrophages, turning them from immunosuppressive to immunostimulatory cells. It also skewed monocyte differentiation from tumor-associated macrophages-like cells to M1-polarized immunostimulatory macrophages. Taken together these data show that IFN-γ overcomes tumor-associated macrophages-induced immunosuppression by preventing the macrophages generation and functions.

Our study shows that in patients with solid tumors and under treatment with paclitaxel and carboplatin, the absolute number of CD8 ($p <0.012$) and the levels of IFN-γ after stimulation with PHA ($p <0.0039$) are parameters that can be considered as prognostic factors for survival.

Similarly, after the end of chemotherapy the percentage of CD4 ($p <0.001$), the rate CD4 / CD8 ($p <0.006$) and the levels of IFN-γ (RIA - $p <0.027$) and IFN-γ (LPS - $p <0.001$) could be considered as highly significant prognostic factors for the survival of cancer patients [17]. Specifically, paclitaxel given one day prior, at the time of initial T cell priming, induced greater numbers of IFN-γ secreting T cells that were associated with tumor growth retardation. Furthermore, altering the sequence of paclitaxel and carboplatin failed to improve and often worsened the antitumor immune effects of the combination, suggesting that enhancing CD8 T cell activity through the improved activation of dendritic cells was one possible mechanism that paclitaxel and carboplatin were improving tumor therapy.

The findings for T cells and cytokines of this study are in agreement with the data published recently in the international literature and provide a promising prospect for their use in the treatment and therapeutic strategy of cancer patients [18-20].

Conclusion

Chemotherapy remains the mainstay of treatment for both early stage as well as metastatic tumors. The immune response against the tumor may be a promising target, especially after much recent data have associated various factors (e.g. number and the type of lymphocyte populations, the production and the action of cytokines, the effects from the combination of chemotherapeutic agents, the induced toxicity etc) with prognosis. The benefits in overall survival with the use of paclitaxel and carboplatin is that this combination has an important impact on the immune response and the induced toxicity. There is still much to clarify regarding the mechanisms governing the development of host antitumor response in order to find strategies to augment it. This may be further exploited for the best supportive and/or novel immunotherapy. However, more studies and research are required, so that in the future we can ensure the best possible outcome in the fight against cancer.

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