ORIGINAL ARTICLE

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Cellular Immune Profile in Patients with Non-small Cell Lung Cancer after Weekly Paclitaxel Therapy

Takanori Sako, Naoto Burioka, Kazuhito Yasuda, Katsuyuki Tomita, Masanori Miyata, Jun Kurai, Hiroki Chikumi, Masanari Watanabe, Hisashi Suyama, Yasushi Fukuoka, Yasuto Ueda and Eiji Shimizu

From the Third Department of Internal Medicine, Faculty of Medicine, Tottori University, Yonago, Japan

Correspondence to: Naoto Burioka, Third Department of Internal Medicine, Faculty of Medicine, Tottori University, 36-1 Nishimachi, Yonago 683-8504, Japan. Tel: +81 859 348 105. Fax: +81 859 348 098. E-mail: burioka@grape.med.tottori-u.ac.jp

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Paclitaxel is a new agent for advanced non-small cell lung cancer (NSCLC). Weekly doses may enhance antitumor activity while minimizing toxicity, but little is known about immune recovery. Paclitaxel (80 mg/m^2) was administered to 10 patients with NSCLC, weekly during 3-week cycles. Natural killer (NK) activity, $CD3^-CD16^+CD56^+$ NK cells, and differential counts were monitored. NK activity appeared in all patients after treatment with paclitaxel therapy. NK activity showed a 27$\pm$9% decrease (mean$\pm$SE) on protocol day 8 and a 37$\pm$7% decrease on day 15 ($p < 0.05$) recovering to 89$\pm$5% of baseline on day 29. With weekly paclitaxel, a decrease in NK cell function persisted through the first cycle but then recovered. Weekly paclitaxel may be less immunosuppressive than agents such as cisplatin.

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Paclitaxel is a tubulin-binding substance extracted from the bark of the Pacific yew, *Taxus brevifolia.* Other tubulin-binding agents such as vinca alkaloids promote microtubule disassembly; in contrast, paclitaxel shifts the equilibrium toward microtubule assembly and stabilizes microtubules by preventing depolymerization (1). In addition, paclitaxel blocks dividing cells at the G_2/M phases of the cell cycle. Paclitaxel has demonstrated clinical activity against solid tumors (2–4). Recently, weekly doses of paclitaxel have been reported to represent a well-tolerated practical administration schedule (2–6). Weekly administration allows treatment with high doses and limits toxicity for normal cells. Cellular cytokinetic considerations imply that frequent exposure of cancer cells to cytotoxic agents at brief intervals affords less opportunity for regrowth of drug-resistant clones. Weekly paclitaxel therapy is considered high-dose, and such therapy also provides sustained cumulative exposure (5). Moreover, weekly doses are less toxic than more frequent administration, so the decrease in granulocytes is less severe. Chemotherapy can therefore be administered at low cost on an outpatient basis (6).

Natural killer (NK) cells are a subpopulation of lymphocytes that display spontaneous cytotoxicity against a variety of targets such as malignant or virus-infected cells irrespective of major histocompatibility antigens (7). In vivo administration of cisplatin-based chemotherapy reduces the NK cytotoxicity of peripheral blood mononuclear cells (PBMC) to almost undetectable levels (8). Therapeutic doses of paclitaxel and docetaxel have been reported to suppress NK cells in vitro. In a recent report, there was a greater decrease in NK cytotoxic activity in patients treated with docetaxel than in patients receiving treatment with paclitaxel (9). However, NK cytotoxic activity and immune recovery with weekly doses of paclitaxel alone have not been reported. The following observations in 10 patients with advanced non-small cell lung cancer (NSCLC) describe NK activity and recovery with weekly doses of paclitaxel.

MATERIAL AND METHODS

Patients

Our studies included 10 patients (7 men, 3 women) with histologically proven NSCLC (mean age, 67 years; range, 42–78). Patient characteristics are listed in Table 1. Seven tumors were adenocarcinomas and three were squamous cell carcinomas. Four patients had locally advanced disease according to the Japan Lung Cancer Society classification (stage IIIa in 1 patient, stage IIIb in 3). Six patients had stage IV disease (2 with lung metastasis and 4 with bone or brain metastasis). Laboratory criteria for protocol entry

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Table 1

Patient characteristics

Age (years)	Gender	History	Stage	Previous therapy	Reason for therapy	Target legion	Total dosage of PTX (times)	Response data	Reason for stopping therapy
66	M	Ad	IV (lung)	No	First-line	RUL	9	PR	Pericardial effusion
76	F	Ad	IIIa	No	First-line	RLL	9	PD	Liver metastasis
78	F	Ad	IV (lung)	No	First-line	RLL	9	PR	Depression
77	M	Sq	IIIb	Operation +UFT	Adjuvant	Tumor marker	3	SD	Interstitial pneumonia
42	M	Ad	IIIb	Operation	Recurrence	Mediastinum Lymph node	6	PD	Obstructive pneumonia
72	M	Sq	IIIb	No	First-line	LUL	6	SD	Polyneuropathy
50	M	Ad	IV (brain)	Operation +γknife	Recurrence	RML	6	PR	Polyneuropathy
66	F	Sq	IV (bone)	No	First-line	RIL	4	SD	Hemorrhagic gastric ulcer
69	M	Ad	IV (brain)	No	First-line	RLL	4	SD	Deteriorating general condition
70	M	Ad	IV (brain)	Operation +γknife	Recurrence	Pleural effusion	4	SD	Deteriorating general condition

Abbreviations: PTX = Paclitaxel; Sq = squamous cell carcinoma; Ad = adenocarcinoma; UFT = Uracil plus Tegafur; RUL = right upper lobe; RML = right middle lobe; RLL = right lower lobe; LUL = left upper lobe; PR = partial response; PD = progressive disease; SD = stable disease

required a granulocyte count of over 1500/μl and a platelet count of over 100 000/μl. One patient with advanced cancer had received prior, relatively mild, chemotherapy (UFT (Uracil plus Tegafur) 400 mg/day). This patient had recovered from the myelosuppressive effects of this therapy, as demonstrated by the differential white blood cell count. The local ethics committee approved the protocol and all the patients gave written informed consent to participate in the study. To judge the therapeutic effect of weekly paclitaxel (WPTX) therapy we examined the brain, chest and abdomen with contrast computed tomography (CT) on the 28th day of each cycle of chemotherapy. We also examined the chest radiographs every week of treatment.

Treatment regimen

Paclitaxel (80 mg/m^2) was diluted in 250 ml of 5% dextrose and administered weekly as a 1-h intravenous infusion on day 1, day 8 and day 15. One cycle of therapy consisted of 3 weeks of treatment. To prevent allergy, all patients were premedicated with dexamethasone (20 mg intravenously 30 min before receiving paclitaxel); ranitidine (50 mg intravenously 30 min before paclitaxel); and diphenydramine (50 mg orally, 30 min before paclitaxel). After an interval of one week, on day 29, if the white blood cell (WBC) count was more than 2 500/μl, the next cycle of chemotherapy was started. Therapy was continued until rapid progression of disease or unacceptable toxicity occurred. In all, the patients received from 3 to 9 dosages of PTX.

Immune monitoring

Immune monitoring was carried out on each dosage of paclitaxel. The baseline immune profile of our patients, established prospectively before weekly paclitaxel treatment, was initiated on the first day (Table 2). The monitoring was done on days 1, 8, 15 and 29 (first administration of the second cycle of chemotherapy). Thus, we were able to follow immune parameter evolution for changes related to therapy throughout the period of treatment.

Tumor cell lines

Cultured cells K562 were obtained from the American Type Culture Collection (ATCC). K562, an erythroleukemic cell line, was maintained in complete RPMI-1640 culture medium, (Gibco BRL, Grand Island, NY) containing 10% fetal calf serum, 100 units/ml penicillin, and 100 μl streptomycin in a 5% CO_2 atmosphere at 37°C.

Cytotoxicity assay

^{51}Cr release cytotoxic assays have been extensively used in the evaluation of NK cell activity for the direct detection of target cell lysis (10–12). ^{51}Cr release cytotoxic assays were performed as described elsewhere (13) with K562 as target cells. One million of these cells were treated with 3.7 Mbq of ^{51}Cr for a period of 1 h at 37°C. Unincorporated ^{51}Cr was removed by repeated washing with Roswell Park Memorial Institute. Radiolabeled tumor cells were plated with effector NK cell populations in 96 well plates (Costar, Cambridge, MA) at effector-to-target cell ratios (E:T ratio) of 10:1, 20:1

Table 2

Immune cell profile of patients before weekly paclitaxel treatment

	Range	Mean ± SE
Leukocytes ($\times 10^6$/ml)	3.8–12.1	7.81 ± 0.89
Neutrocytes ($\times 10^6$/ml)	2.59–9.39	5.76 ± 0.66
Lymphocytes ($\times 10^6$/ml)	0.57–3.54	1.63 ± 0.34
Monocytes ($\times 10^6$/ml)	0.37–1.31	0.66 ± 0.98
%CD3$^-$CD16$^+$CD56$^+$ T cells (%)	2.92–24	6.94 ± 3.82
%CD3$^+$CD4$^+$ T cells (%)	29.4–59.5	45.0 ± 4.92
%CD3$^+$CD8$^+$ T cells (%)	17–39.5	26.7 ± 2.78
NK cell cytotoxic activity (%)	18–43	30.7 ± 0.57

%CD3$^-$CD16$^+$CD56$^+$ T cells, % of CD3$^-$CD16$^+$ CD56$^+$ T cells in peripheral blood lymphocytes.
%CD3$^+$CD4$^+$ T cells, % of CD3$^+$CD4$^+$ T cells in peripheral blood lymphocytes.
%CD3$^+$CD8$^+$ T cells, % of CD3$^+$CD8$^+$ T cells in peripheral blood lymphocytes.
Abbreviations: NK = natural killer; SE = standard error.

and 40:1 (the data of E:T ratio 10:1 and 40:1 not shown) and incubated for 3.5 h at 37°C. Cells were centrifuged at 1 500 rpm for 8 min followed by removal of 80 μl supernatant aliquots of triplicate cultures. Release of ^{51}Cr into the supernatant was counted with a gamma scintillation counter (ARC-300; Aloka, Tokyo, Japan). Cytotoxic ability of effector cells to lyse tumor targets was calculated as:

%cytotoxicity
= (experimental release–spontaneous release)/
(maximum release–spontaneous release)×100 (%)

Maximum ^{51}Cr release from tumor cells lines was determined by adding 100 μl of 1 N hydrochloric acid, while the spontaneous ^{51}Cr release was assessed by adding 100 μl of complete RPMI medium.

Immunophenotype analysis

CD4$^+$, CD8$^+$, and CD3$^-$CD16$^+$CD56$^+$NK cells, and the differential w.b.c., were examined with every administration of paclitaxel. To calculate the total number of lymphocytes, monocytes and granulocytes, we used the hematology analyzer (Beckman Counter; NY, USA) and to obtain their rate we counted the 200 cells in May-Gimsa stain. Frequency distributions of CD4$^+$ cells, CD8$^+$ T cells and CD16$^+$CD56$^+$ NK cells were determined with fluorochrome-labeled monoclonal antibodies (Exalpha Biologicals, I Boston, MA) that recognized CD4$^+$, CD8$^+$ and CD3$^-$CD16$^+$CD56$^+$. Monoclonal antibody 50 μl was added to 100 μl of whole blood and incubated for 20 min at 20°C. After lysing whole blood with Optilyse B (Immunotech; Marseille, France), the specimen was washed with phosphate buffered saline (PBS) and the white cells were fixed with 0.5% paraformaldehyde in PBS. An analysis to identify surface antigen expression was carried out in 10 000 cells by flow cytometry (Beckton Dickinson FACScan; Sandiego, CA).

Data analysis

For assessment of differences, we used a two-way analysis of variance (ANOVA). Multiple comparisons between dosage days were performed with Sheffé's test (Statview; Abacus Concepts, Berkley, CA). Differences were considered significant at $p < 0.05$.

RESULTS

Of the 10 patients in the study, 3 received 9 doses of PTX; 3 received 6 doses of PTX; 2 stopped treatment at 6 doses because of grade 2–3 polyneuropathy, and 2 patients stopped treatment after 4 doses because of deteriorating general condition. Patients who received 9 doses of therapy showed a partial response with respect to the primary lesion. However, one patient developed multiple liver metastases and stopped treatment. In another patient, therapy was stopped because of cardiac tamponade caused by carcinomatous pericarditis and was restarted after pericardiocentesis. The last patient did not continue treatment because of depression. None of the patients interrupted or stopped therapy because of immunosuppression or infection during chemotherapy.

We examined the differential WBC and NK cell activity in the first and second cycles of treatment. Leukocytes tended to decrease in all patients after treatment with paclitaxel (Fig. 1). On day 1 the leukocytes were 7.81 ± 0.89 ($\times 10^6$/ml) (mean ± SE) and decreased to 4.6.3 ± 0.60 ($\times 10^6$/ml) (mean ± SE) on day 8 and 4.72 ± 0.38 ($\times 10^6$/ml) (mean ± SE) on day15. Patients receiving weekly paclitaxel showed a 15 ±7% (mean ± SE) decrease in leukocyte count on day 8 ($p < 0.05$) and a 37 ± 5% (mean ± SE) decrease on day 15 ($p < 0.05$). Neutrophils had decreased significantly on the 8th day after initiating paclitaxel treatment ($p < 0.05$) and also on the 15th day ($p < 0.05$). Monocytes showed a significant decrease on days 8 and 15 ($p < 0.05$). Lymphocytes decreased on the 8th day, and began to recover on day 15. A nadir was observed after the third dose (from day 15 to day 22). In the second

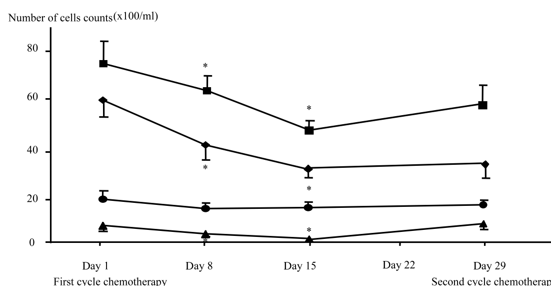

Fig. 1. Cell numbers for leukocyte subsets during weekly paclitaxel treatment. Mean values (±SE) leukocyte subsets for all patients are shown; * $p < 0.05$ compared with the day 1 value, by multiple comparisons performed with Sheffé's test. Total leukocytes: –■–; total neutrocytes: –◆–; total lymphocytes: –●–; total monocytes: –▲–.

cycle of chemotherapy, commencing after a withdrawal period of one week, leukocytes recovered to 87% of the day 1 baseline. Loss of lymphocytes also recovered during second cycle of chemotherapy.

When cells were stained with trypan blue, viability of K562 was always above 90%. When the reproducibility of ^{51}Cr-release assays with K562 was confirmed, a variation of inter-assay, which was measured in NK activity of same patient on different days without the treatment, was 6.5%. NK activity decreased in all patients after treatment with paclitaxel. Because baseline NK activity varied greatly between individuals, we presented NK activity after paclitaxel treatment as a ratio to the baseline value [$(NK_{post}/NK_{pre}) \times 100\%$] (Fig. 2). NK activity showed a 27%±9% (mean±SE) decrease from the day 1 value on day 8 and a 37±4% decrease on day 15 ($p < 0.05$), and recovered to 89±5% (mean±SE) of baseline on day 29 (at the start of the second cycle of chemotherapy). $CD3^-CD16^+CD56^+$ NK cells showed a tendency towards decrease on day 15, and almost recovered by day 29 (Fig. 2).

DISCUSSION

Our study demonstrated decreased NK activity but rapid immune recovery with weekly doses of paclitaxel as a single agent. With chemotherapy administration, leukocytes, neutrophils, $CD3^-CD16^+CD56^+$ cells, and NK activity all decreased. We observed a decrease in NK activity in all patients after treatment with paclitaxel, but this soon recovered, reaching 89±5.3% of baseline during the next cycle.

Previous studies indicated NK activity as an important element in tumor immunosurveillance (14). Cytotoxic drug therapy has a negative effect on immune reactivity. Patients show a decrease in NK activity even after surgery (15, 16). Results of immune monitoring suggest a significant progressive reduction in NK cell activity during combined-agent adjuvant chemotherapy, despite the relative stability of lymphocyte subsets. Cisplatin-based combined chemotherapy persistently, strongly depletes NK cell function, and quantitative recovery of lymphocytes does not necessarily indicate qualitative recovery of immune function. One month after the last cycle of treatment with cisplatin-based combined chemotherapy, NK activity was still reduced to one-third of the initial mean value (17). Moreover, with radiotherapy in combination with cisplatin-based chemotherapy, a severe reduction of cytotoxic activity was observed for at least three months following initiation of treatment (18). Certain drugs such as melatonin may protect PBMC against the immunosuppression of cisplatin-based chemotherapy (19). In postoperative chemotherapy for gastrointestinal cancer with mitomycin C, NK cell activities were significantly decreased on day 7 (from 31.4% to 16.6%) and remained decreased on day 21, to 18.6% (20).

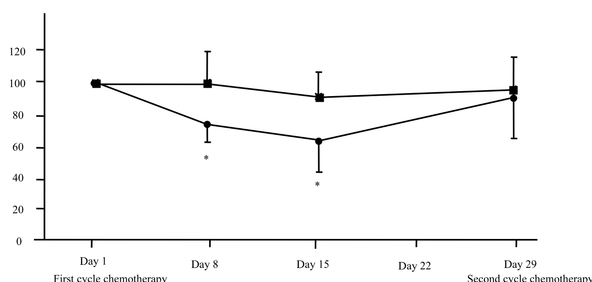

Fig. 2. Natural killer cell (NK) activity and cell number for $CD3^-CD16^+CD56^+$ NK cells during weekly paclitaxel treatment. NK activity and $CD3^-CD16^+CD56^+$ NK cell number for each patient after weekly paclitaxel treatment was expressed relative to the individual baseline value before chemotherapy [$(NK_{post}/NK_{pre}) \times 100\%$]. Mean values (±SE) for all patients, relative NK activity and for $CD3^-CD16^+CD56^+$ NK cells are shown; * $p < 0.05$ compared with the day 1 value, by multiple comparisons performed with Sheffé's test. Relative NK activity (%): –●–; relative total number of $CD3^-CD16^+CD56^+$cell (%): –■–.

Some anti-cancer drugs do not decrease NK activity in vitro (21). Paclitaxel is known to cause polymerization of microtubules and to alter many cellular functions. Recent studies demonstrated that a low dose of paclitaxel did not significantly affect the ability of NK cells to lyse K562 cells (22). In a previous in vitro study, paclitaxel mimicked the action of lipopolysaccharide (LPS) on murine macrophages by enhancing LPS signaling via TLR4 (23).

In vivo, immune function was decreased more significantly after treatment with docetaxel than after paclitaxel, and the loss of immune function with paclitaxel treatment was slight (9). In patients with advanced breast cancer, docetaxel demonstrated a more marked decline in NK activity than with paclitaxel (24).

In summary, weekly paclitaxel therapy caused persistent depletion of NK cell function during the first cycle of chemotherapy, but NK activity recovered at the start of the second cycle. Immune suppression through weekly paclitaxel therapy may be less than that with combined chemotherapy including cisplatin. Weekly paclitaxel could contribute towards a lower risk of infection and a reduction in the use of antibiotics in the treatment of lung cancer.

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