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ORIGINAL ARTICLE

Effect of dendritic cell/cytokine-induced killer cell immunobiological cancer therapy combined with adjuvant chemotherapy in patients with triple-negative breast cancer*

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Abstract

Objective The aim of the present study was to investigate the effect of dendritic cell (DC)/cytokine-induced killer cell (CIK) immunobiological cancer therapy in patients with triple-negative breast cancer (TNBC) who underwent adjuvant chemotherapy.

Methods From January 2010 to October 2013, 120 patients with postoperative TNBC were recruited and included in the study. Patients were enrolled in one of two groups according to whether they accepted DC/CIK immunobiological cancer therapy during adjuvant chemotherapy; the patients in the DC/CIK group underwent adjuvant chemotherapy combined with DC/CIK immunobiological cancer therapy, and the control group underwent adjuvant chemotherapy alone. When six cycles of adjuvant chemotherapy and six cycles of DC/CIK immunobiological cancer therapy had been completed, differences between the two groups with regard to quality of life (QoL), immunological indicators (CD3, CD4, CD8, and NK cell levels), disease-free survival (DFS), and side effects of chemotherapy and DC/CIK treatment were evaluated.

Results In the DC/CIK group, the proportion of NK cells and $CD3^+$ and $CD4^+$ T-cell subgroups significantly increased, and the proportion of $CD8^+$ cells decreased when they were compared before and after DC/CIK therapy ($P < 0.05$). However, there were no significant changes in the control group. By the final follow-up, DFS of the treatment group and the control group was 38.4 and 34.2 months, respectively. The QoL improved in the patients treated with chemotherapy plus DC/CIK therapy compared with the patients treated with chemotherapy alone, and the difference between groups was significant ($P < 0.05$). The side effects of two groups were tolerable and not significantly different between the two groups.

Conclusion The DC/CIK treatment had potential benefits for patients with TNBC compared with the control group, and was not associated with any obvious side effects. Therefore, DC/CIK therapy is a safe and effective method for the treatment of TNBC.

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Breast cancer has become one of the leading causes of death among female cancer patients, and morbidity and mortality due to this disease are still rapidly rising [1]. Treatments for breast cancer include surgery, radio-

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therapy, chemotherapy, endocrine treatments, targeted molecular therapy, and biological treatments. However, immunohistochemical studies investigating triple-negative breast cancer (TNBC), which accounts for approximately 30% of breast cancers, reveal that the estrogen receptor and progesterone receptor are each present in < 10% of cases, and human epidermal growth factor 2 (Her-2) is not expressed (as indicated by fluorescent *in-situ* hybridization tests). Therefore, specific treatments options for TNBC are limited. Dendritic cell (DC)/cytokine-induced killer cell (CIK) treatment is expected to be an effective method, in addition to surgery, chemotherapy, and radiotherapy. As a novel treatment measure, DC/CIK immunobiological cancer therapy has the advantage of minimal cytotoxicity and highly specific targeting of cancer cells compared with surgery, radiotherapy, and chemotherapy. Studies have demonstrated that DCs are the most powerful antigen-presenting cells identified to date. They play an important role in regulating the body's immune response. Furthermore, as initiating factors of the immune response, DCs play a key role in the production and maintenance of tumor immunity [2]. CIKs have the advantages of rapid proliferation, high tumor-killing activity, broad-spectrum tumor-killing activity, and low toxicity relative to normal hematopoietic precursor cells in bone marrow; they also remain effective against multidrug-resistant tumor cells [3]. Co-culture of DCs and CIKs causes CIKs, which are regulated by DCs, to directly kill tumor cells. They exhibit both the strong anti-tumor activity of T lymphocytes and the restrictive tumor-killing characteristics of non-MHC natural killer (NK) cells [4]. In addition, DC/CIK immunobiological cancer therapy may enhance quality of life (QoL) [5]. Therefore, immunobiological cancer therapy for the treatment of TNBC may be useful to supplement current comprehensive treatment.

Patients and methods

Clinical data

Patients with stage Ib, II or III TNBC diagnosed by pathology or cytology examination at the General Hospital of Shenyang Military Region (China) were included in the present study. Inclusion criteria were as follows: age 18–75 years, Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, white blood cell count > 4.0×10^9/L, platelet count > 100×10^9/L, and an expected survival time of more than 12 weeks. Exclusion criteria were as follows: other tumors, uncontrolled cardiac disease, liver and renal insufficiency, progressive brain metastases, uncontrolled third-space fluid collections, pregnancy or breast-feeding, or a mental or psychological disease preventing cooperation with treatment and curative effect evaluation. The study was approved by the Ethics Committee of the General Hospital of Shenyang Military Region, China. Distribution between the groups was balanced ($P > 0.05$), as shown in Table 1.

Table 1 Baseline characteristics of the overall population (*n*)

Characteristic	*n*	DC/CIK group	Control group	*P*
Median age (year; range)		52.5 (35–73)	54.3 (33–73)	1
Disease stage				0.83
I	12	5	7	
II	79	40	39	
III	29	15	14	
Surgery				0.54
Modified	108	55	53	
Conservative	12	5	7	
ECOG performance status				0.53
0	100	50	50	
1	20	10	10	
CE + TC regimen	120	60	60	1
Menstrual status				0.19
Premenopause	67	37	30	
Postmenopausal	53	33	30	

Methods

A total of 120 patients were divided into two groups according to whether they accepted DC/CIK immunobiological cancer therapy. Sixty patients in the DC/CIK group received adjuvant chemotherapy combined with DC/CIK immunobiological cancer therapy, and 60 patients in the control group received adjuvant chemotherapy alone. Blood samples were collected from each patient in the DC/CIK group 1–3 days before each chemotherapy cycle started. Lymphocytes were separated from blood samples, cultured *in vitro*, and subsequently administered to the patients (via subcutaneous injection for DCs and intravenous injection for CIKs). The control group was regularly assessed and followed to determine disease progression. Blood samples of all patients were analyzed before and after DC/CIK reinjection.

Preparation of CIK and DC

Peripheral blood mononuclear cells of patients were separated, and auto-CIKs were generated as described previously [6–7]. After 7 days of culture, CIK cells were co-cultured with stimulated DC for another 7 days. CIK cells were harvested and analyzed to determine the phenotype, cytotoxicity, and secretion of cytokines. Safety tests were performed during the course of cell culture. All products were free of bacterial and fungal contamination, negative for mycoplasma, and contained < 5 Eu endotoxins.

Evaluation criteria

Efficacy and toxicity: A detailed physical examination was performed to determine health condition (based on the ECOG score), and routine blood tests, liver and kidney

Table 2 Variation in NK cells, T-cell subgroups before and after treatment (means ± SD, n = 120)

Groups	$CD3^+$	$CD4^+$	$CD8^+$	NK	P
DC/CIK					< 0.05
Before treatment	47.3 ± 10.2	33.1 ± 10.3	32.2 ± 10.7	16.5 ± 3.8	
After treatment	60.2 ± 12.1	40.2 ± 9.5	27.5 ± 5.3	26.2 ± 7.2	
Control					> 0.05
Before treatment	48.3 ± 10.6	31.8 ± 8.3	32.8 ± 9.8	15.4 ± 4.5	
After treatment	47.3 ± 12.5	32.4 ± 9.7	33.3 ± 12.5	16.9 ± 4.6	

Table 3 QoL questionnaire of the two groups (n)

Groups	QoL (n)			P
	High	Medium	Low	
DC/CIK	19	29	12	0.036
Control	13	22	25	

function tests, and electrocardiography were performed. The side effects of toxicity were divided into levels 0 to IV according to the 1981 uniform standards of the World Health Organization. Disease-free survival (DFS) refers to the time interval from the first day after surgery to the first recurrence or metastasis time. The follow-up time of the study was until October 2014. A scoring system was adopted to assess the QoL questionnaires; 24 points represented the best QoL and 80 points represented the worst QoL. When patients were discharged, an improvement in QoL was considered to be a reduction by more than 24 points, and a decline in QoL was considered to be an increase by more than 24 points.

Statistical analysis

Data were analyzed using SPSS 19.0 statistical software. Variance analysis and Pearson's χ^2 test were used to assess the differences in clinical characteristics between the two groups. Kaplan-Meier analysis was used to compare DFS between the two groups. Descriptive statistics were used for the evaluation of safety. $P < 0.05$ was considered to be statistically significant.

Results

With regard to the variation in NK cell and T-cell subgroups in the DC/CIK group, the proportion of NK cells and $CD3^+$ and $CD4^+$ T-cell subgroups significantly increased, and the proportion of $CD8^+$ cells decreased when they were compared before and after DC/CIK immunobiological cancer therapy ($P < 0.05$). However, there were no significant changes in the control group (Table 2). By the final follow-up, DFS of the treatment group and the control group was 38.4 and 34.2 months, respectively. DFS of the treatment group was extended by 4.2 months, as shown in Fig. 1.

The QoL improved in the patients treated with chemotherapy plus DC/CIK immunobiological cancer therapy compared with the patients treated with chemotherapy alone, and the difference between groups was significant ($P < 0.05$), as shown in Table 3. The main side effects of chemotherapy were alopecia, bone marrow suppression, and gastrointestinal reactions. More than 75% of the patients in the two groups appeared to have alopecia. The incidence of leukopenia in the two groups was more than 70%; leukopenia was relieved by injection of human granulocyte colony-stimulating factor (G-CSF). Water/sodium retention and allergic reactions were not observed in either of the two groups. Gastrointestinal reactions did not exceed III degrees. Patients in the DC/CIK group experienced fevers, but temperatures were below 38.5 ℃ and returned to normal within 24 h without specific treatment. The side effects of two groups were tolerable and not significantly different between the two groups. Detailed data were presented in Table 4.

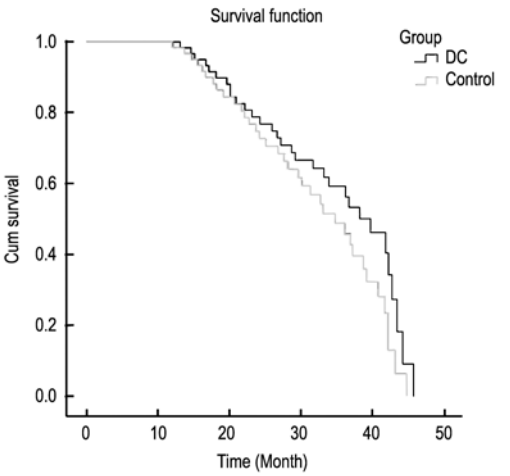

Fig. 1 Kaplan-Meier estimates of disease-free survival between the two groups

Discussion

Breast cancer is the most common malignant tumor among women; it has a high degree of heterogeneity, and has become a significant cause of morbidity and mortality worldwide. In recent years, the incidence of breast cancer

Table 4 Side effects of the treatments in the two groups (*n*)

Side effects	DC/CIK group (*n* = 60)						Control group (*n* = 60)					
	0	I	II	III	IV	%	0	I	II	III	IV	%
Alopecia	14	20	19	7	0	76.7	13	18	14	15	0	79.2
Leukopenia	17	14	24	5	0	72.0	15	17	20	8	0	75.0
Thrombocytopenia	41	14	5	0	0	31.7	40	15	5	0	0	33.3
Nausea and vomiting	38	15	5	2	0	36.7	34	12	7	7	0	43.3
Liver damage	50	10	0	0	0	16.7	47	13	0	0	0	21.7
Renal damage	55	5	0	0	0	8.33	53	7	0	0	0	11.7
Neurotoxicity	53	7	0	0	0	11.7	50	10	0	0	0	16.7
Fever	51	9	0	0	0	15.0	58	2	0	0	0	3.33

is increasing and it affects patients at increasingly young ages [8]. TNBC, an important subtype of breast cancer, is generating considerable attention because of its high level of invasiveness and risk of metastasis. However, current treatment options for TNBC are limited; thus, researchers are attempting to identify new methods. DC/CIK immunobiological cancer therapy is a treatment method in which lymphocytes are isolated from blood samples collected from the patients themselves, inductively amplified *in vitro*, and subsequently re-injected into the body. This therapy is not associated with gastrointestinal reactions or bone marrow toxicity, unlike cytotoxic drugs. In the DC/CIK group, significant changes in the numbers of $CD3^+$, $CD4^+$, $CD8^+$, and NK cells before and after treatment were observed ($P < 0.05$), whereas changes in the control group were not significant. DCs uptake enthetic antigens in the form of receptors and combine with the MHC I and II molecules on these antigens to stimulate activation of primary-type $CD8^+$ T cells and $CD4^+$ T cells. In addition to inducing antigen-specific cytotoxic T lymphocytes, DCs can directly or indirectly influence B cell proliferation and activate the humoral immune response [9]. DCs are mainly antigen-presenting cells. Their ability to capture, process, and present antigens in MHC class I and II and their expression of co-stimulatory molecules for T lymphocytes together with the release of proimmune response cytokines are central to the initiation of an efficient adaptive immune response. Studies have shown that cytotoxicity significantly increases after DC stimulation of CIKs [10]. The tumor inhibition rate is significantly higher for co-cultured DC-CIKs than for CIKs (62.9% vs. 41.5%; $P < 0.05$) [11]. DCs and CIKs can promote the antitumor effect of chemotherapy drugs. Co-cultured DCs and CIKs can generate a new cell population, and their cytotoxicity and cell proliferation activity are higher than those of CIKs [12]. Antigen-sensitized DCs co-cultured with CIKs and MDA-MB-231 cell lysates have a stronger killing activity than do separate CIKs and non-sensitized DC-CIKs ($P < 0.05$). Therefore, the combined application of CIKs and DCs is synergistic for breast cancer treatment, exhibiting high killing activity and antigen presentation ability. DC/CIK biological immunotherapy was not associated with significant toxic reactions, including bone marrow toxicity and gastrointestinal reactions (nausea and vomiting). In the present study, the common side effects observed in the DC/CIK group were alopecia, bone marrow suppression, and gastrointestinal reaction, and these were consistent with the results of several studies investigating combination chemotherapy. After treatment of these symptoms, side effects were resolved and patient tolerance was acceptable.

In addition, side effects were significantly lower in the DC/CIK group than in the conventional intravenous chemotherapy group. In the present study, the QoL of the combination chemotherapy group was higher than that of the control group, and the difference was statistically significant. We evaluated the clinical effects of chemotherapy plus DC/CIK and chemotherapy alone. DFS of the DC/CIK group was longer than that of the control group, and the difference was significant. The study showed that the effective rate of the combination group was higher than that of the single treatment group. Therefore, the clinical efficiency of DC/CIK therapy was confirmed and is worthy of future consideration.

The efficiency of DC/CIK therapy was confirmed in the present study, and DC/CIK biological immunotherapy should be considered a worthy treatment option. Collectively, the results of the present study demonstrated that reasonable application of DC/CIK immunobiological cancer therapy effectively improved QoL in TNBC patients. Moreover, DC/CIK immunobiological cancer therapy was well tolerated and led to a significantly improved immune state, making it an appropriate treatment option for TNBC patients who do not progress after initial induction therapy. Therefore, application of DC/CIK immunobiological cancer therapy had potential clinical benefits for TNBC patients.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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