

Clinical effects of immunotherapy of DC-CIK combined with chemotherapy in treating patients with metastatic breast cancer

Qixin Mao, Lianfang Li, Chongjian Zhang, Yadong Sun, Shanqing Liu and Shude Cui*

Department of Breast, Henan Cancer Hospital, Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, China

Abstract: This study aimed to analyze the clinical effects of dendritic cell (DC) and cytokine-induced killer (CIK) immunotherapy combined with chemotherapy on patients with metastatic breast cancer. Twenty patients were included into this study who were diagnosed as metastatic breast cancer (MBC). DC and CIK were augmented by in vitro culture and then rein fused into body through vein. The pain relief rate (RR), toxic and side effects of chemotherapy, immunity functions and living quality of patients were observed. DC and CIK cells were induced by the autologous peripheral blood mononuclear cells (PBMC). Meanwhile, flow cytometry was used to measure T cell subsets and natural killer T (NKT) cells in patients in the two group before and after the biological treatment. After DC and CIK were rein fused into the patients body, no severe side-effect was found. It was also found that cellular immunotherapy combined with chemotherapy the immunotherapy of cells improved the immunity, the living quality of patients and the disease control rate (DCR). In conclusion, cellular immunotherapy produces small side effects; it combined with chemotherapy is able to improve the DCR and living quality of patients and prolong their lives.

Keywords: DC; CIK; Metastatic breast cancer; Immunotherapy of cells.

INTRODUCTION

Breast cancer, one of the most common malignant tumor occurring in female, is one of the leading causes for the death of female; gene, hormone, age and environment are all the related hazards of the occurrence of breast cancer [1]. (Soon Wong *et al.*, 2009). The treatment of breast cancer covers surgery and non-surgery comprehensive therapy (Hongjiang, 2011). With the progress of theoretical research on tumor cells, (Palucka *et al.*, 2011), immunotherapy has become the focus in the treatment of breast cancer.

Zhao Qingye, *et al.* observed the level of IFN- γ , IL-10 and IL-12 after carrying out therapy of DC combined with chemotherapy for treating the advanced gastroenteric tumor and found that DC was effective in alleviating the side-effects of chemotherapy, improving the immunity functions and living quality of patients (Qingye *et al.*, 2011). Wang Bing, Wang Hongmei *et al.* found that the functions of DC was closely related to its maturity and the mature DC played an important role in the immunity of tumor (Bing *et al.*, 2013). Jiang Jingting used the tumor bearing mice model to make a preclinical study of CIK and it was revealed that CIK had obvious antitumor effects both in the solid tumor and the tumor in blood system. Moreover, based on clinical practice, he proved that CIK could be applied in treating several malignant tumors and it was effective in prolonging the lives of patients without any side-effects (Jingting, 2012). In study from Zhong Guocheng, Jing Xinrong *et al.*, it was found that after the co-culture of Ag-DCs and CIK, the killing

activity of CIK was activated in A549 cell and autologous tumor cells; the combination of Ag-DCs with CIK was effective in curing lung adenocarcinoma, improving the living quality and the clinical responses. Based on the studies above, immunotherapy of DC-CKI has a great potential effect on treating the tumors and it is a therapy with special efficacy in treating metastatic breast cancer (Guocheng *et al.*, 2010). Twenty patients diagnosed as metastatic breast cancer were selected, and then they received the chemotherapy as per plan TA, followed by immunotherapy of DC-CKI. Besides, the curative effect was assessed in terms of adverse reaction, side-effect and cellular immune effect.

MATERIALS AND METHODS

Objects

Twenty patients who were admitted into The 452 Hospital of Chinese People's Liberation Army from February 2011 to October 2012 were selected as the research objects. All patients were diagnosed as MBC by histology or cytology. They ranged from 40 to 70 years (mean 45 \pm 5 years). Karnofsky performance status score (KPS) of patients were no less than 60 and they were estimated to live for three months at least. All patients have not received any treatment for tumor within the recent two months. Indicators of blood routine examination and hepatic and renal function were all kept normal. Besides, it also made a statistical analysis on several important factors affecting the prognosis, such as age, grade, pathologic type and metastatic positions. Patients with incomplete heart, liver and kidney functions, other malignant tumors at the same time and patients or psychological disease were excluded.

*Corresponding author: e-mail: csd@ibhsedu.com

Preparation of DC

Blood cell separator (Kabi Fresenius, German) was used to separate PBMC in a quantity of 2×10^9 and 3×10^9 from peripheral blood and meanwhile 80ml plasma was collected. After MC was separated by Ficoll-Hypaque (Gibco, USA), the cells were centrifuged for eight times, and then suspended in serum-free RPMI 1640 (PAA laboratories GmbH, Linz, Austria) to adjust the concentration ranged from 2×10^{10} to 4×10^{10} /ml. Then the cells were placed into 6-well plate in an incubator with 5% CO₂ at 37°C for six days. Next, the plate was shook up and the suspension cells were collected into another 50 ml centrifuge tube. The plate was washed once or twice with serum-free RPMI 1640 culture medium and then add with RPMI 1640 culture solution containing 10% FBS for further culture. In the third day, 2 or 3ml DC culture solution was added into each well. In the fifty-day, tumor specific antigens were added (the total concentration ranged from 20 to 80µg/ml). After 12 or 16 hours, tumor necrosis factor (TNF- α , 500U/ml) was added. In the eighth day, the cells collected were centrifuged. After washing by normal saline thrice and resuspended by normal saline containing 10% plasma, the cells was rein fused through vein for four times in a volume of 100ml between two DC rein fusions.

Preparation of CIK

MC separated by the Ficoll-Hypaque (Gibco, USA) was suspended in the RPMI 1640 culture solution containing 10% FBS to adjust the concentration to 2×10^6 /ml and then placed into the culture bottle. After γ -interferon (INF- γ , 1000U/ml) was added, the liquid was placed in the incubator with 5% CO₂ at 37°C for 24h. In the next day, CD3 McAb (0.5µg/ml) and interleukin-2 (IL-2, 1000U/ml) were added for further culture. In the third day, RPMI 1640 culture solution containing 10% FBS was added. In the eleventh or twelfth day, CIK collected was centrifuged and washed by normal saline for three times. Next, the cells were resuspended by the normal saline containing 10% plasma. Finally 100ml liquid obtained was rein fused through vein for four times between two times of DC rein fusions.

Quality control of cells and the activity analysis

In every two days before cells rein fusion, carry out the bacterial culture for bacterial culture. In every one-hour before cell rein fusion, toxin was detected by gel method. Then, Immune activity analysis was performed on DC and CIK collected in the 7th and 12th day respectively. Next, DC was marked with CD3, HLA-DR, CD1a, CD80, CD86 respectively while CIK was marked with CD3 or CD56 for 30min at 4°C in the dark. After two times of washing, the cells were resuspended in phosphate buffer solution (PBS) and then the liquid was put on the device (Caliber BD) for detection.

Analysis of immunity functions in patients before or after reinfusion

Peripheral blood was collected from patients at the day collecting mononuclear cells and the seventh day after treatment respectively. Then CD3, CD4, CD8, CD56 were marked with fluorescence in order to detect T cell subsets.

Therapy

All the patients were examined and scored by KPS scoring. They received chemotherapy as per TA scheme, i.e., in the first three days, orally take dexamethasone 20 mg 12 and 6 h before administration of docetaxel 20mg, once a day in the first day intravenously inject adriamycin 50mg/m² in the second day, add docetaxel injection 75 mg/m² into 250ml normal saline and then intravenously inject for 3h; blood pressure, impulse and breath were closely monitored in the process of injecting docetaxel; meanwhile, anti-emesis processing should be given; G-CSF treatment was given in diapause according to the blood routine 21 days was taken as one period. All patients were collected the PBMC two days before the chemotherapy in each period. In the third day after chemotherapy, 1×10^9 CIK and 1×10^7 DC was suspended with 0.9% normal saline and then the suspension was injected into patients within 1h by eight times for alternatively. When there were severe side-effects beyond resistance or the treatment proceeded for 8 periods, the therapy would be terminated. Every time one period was ended, the QOL (quality of life) and the immunity functions of patients were all evaluated and detected. Moreover, the evaluation of effective rate was performed in every two periods.

Indicators of observation

According to WHO, the effects on solid tumors was divided into complete remission (CR), part remission (PR), stable disease (SD) and progressive disease (PD), among which the CR+PR was applied to calculate efficiency. KPS scoring, a grading system, was applied to evaluate the QOL for patients before and after treatment. Meanwhile, when observing whether there were some side-effects in patients, the chemotherapy responses were divided into degrees from 0 to IV based on the WHO standard. Besides, the reinfusion of DC-CIK in patients were also observed, including some symptom changes like fatigue or weakness, lack of appetite, partial soreness, night sweat and insomnia. It was also observed whether there were side-effects like fever and erythra after the immunotherapy of DC-CIK. The clinical symptoms of patients in two groups before and after the immunotherapy of DC-CIK were observed for comparison.

STATISTICAL ANALYSIS

SPSS17.0 software was applied for data processing and was expressed in mean \pm square error. Besides, t test was used for statistical test.

RESULTS

Adverse reaction

All patients were found with no adverse reactions in hypodermic injection. Except one patient with a slight fever (body temperature reached 37.6°C but restored normal by himself without specific treatment), and the other patients all acted normally in the intravenous infusion. All patients tolerated the treatment.

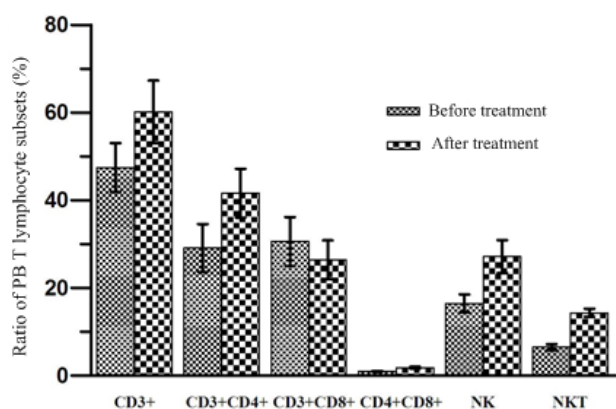


Fig. 1: Changes in cellular immune phenotype before and after immunotherapy of DC-CIK

Therapy results

Twenty patients experienced the combination of DC-CIK immunotherapy of DC-CIK with chemotherapy, among which 3 patients were treated with CR, 12 with PR, 2 with SD, and 3 with PD. The general rate of efficacy was 75%, and the total DCR was 85% (table 1).

Toxic and side effects

Toxic and side effects induced by chemotherapy include hair loss, myelosuppression and gastrointestinal reaction. After receiving treatment, 75% patients in this study started to lost their hair, 50% patients were found with a reduction of white blood cells over II degree. But they were all restored to be normal after treated with G-CSF. However, no patients were found with water-sodium retention or allergy reaction. The gastrointestinal reaction was mostly kept in III degree. All patients tolerated toxic reactions.

Cellular immune response

Before DC-CIK cellular immunotherapy combined with chemotherapy, T lymphocyte (CD3⁺) accounted for 47.46±9.66, T helper cell (CD3⁺/CD4⁺) was 29.12±9.4, T suppressor cell (CD3⁺/CD8⁺) was 30.81±9.58, NK cells (CD3⁺/CD16⁺56⁺) was 16.46±3.57, NKT cells (CD3⁺/CD16⁺56⁺) was 6.53±1.19. But one week after treatment, T lymphocyte (CD3⁺) accounted for 47.46±9.66, T helper cell (CD3⁺/CD4⁺) was 29.12±9.4, T suppressor cell (CD3⁺/CD8⁺) was 30.81±9.58, NK cells was 16.46±3.57, NKT cells (CD3⁺/CD16⁺56⁺) was 6.53±1.19. Based on the analysis of the data above, it was known that, one week

after the treatment, the proportion of total T lymphocyte (CD3⁺), T helper cell (CD3⁺/CD4⁺), NK cells (CD3⁺/CD16⁺56⁺) and NKT cells (CD3⁺/CD16⁺56⁺) obviously rose but an obvious decrease was found in the proportion of T suppressor cell (CD3⁺/CD8⁺), and moreover, the immune function of the patients remarkably enhanced. The detailed results are shown in table 2 and fig. 1.

Table 1: Patients in each efficacy category post-treatment

Efficacy	CR	PR	SD	PD
Patients	3	12	2	3
Ratio	15%	60%	10%	15%

Improvement in symptoms in patients before and after clinical treatment

After receiving the combination of immunotherapy of DC-CIK with chemotherapy, nearly half of patients had some relief from fatigue or weakness, lack of appetite, insomnia and night sweat (table 3).

DISCUSSION

Nearly half of patients with breast cancer (Rongguo *et al.*, 2013) are inclined to be under the risk of palindromia and metastasis. The incidence rate of MBC three or five years after the initial diagnosis reached 75%. At present, the therapy for MBS mainly cover Endocrine therapy, chemotherapy, targeted therapy and other drugs which can be applied in the whole body. As to isolated and limited metastatic foci, radiotherapy and surgical treatment can be considered. Chemotherapy and radiotherapy can produce great side effects on patients, especially for patients with advanced breast cancer; therefore, comprehensive therapy is a new research direction for treating MBC.

DC is a known professional antigen-presenting cell with the strongest functions. DC vaccine can stimulate the body to produce specific cytotoxic T cells immune response against tumor antigen in immunotherapy. Nowadays, DC vaccine applied in the active immunotherapy for specific tumors can be roughly divided into two kinds, i.e., DC polypeptide vaccine and DC gene vaccine (Guozhi *et al.*, 2012). CIK, a heterocyst subset acquired from the co-culture of PBMC and several cell genes in vitro, carries the anti-tumor activity of T cell and the non-MHC restricted cytotoxic activity of NK cell. It is featured by quick proliferation, high activity and broad spectrum in killing tumors. The culture in vitro hints that DC vaccine can effectively improve the activity of CIK in killing tumor cells. CIK (Nian and Huyi, 2014) is a heterocyst subset acquired from the PBMC proliferation of several cell genes in vitro, and T cell CD3⁺CD56⁺ and CD3⁺CD8⁺ are the fundamental effect or cell. Besides, it also carries the strong activity of T lymphocyte and non-major his to compatibility complex

Table 2: Changes in cellular immune phenotype before and at the 7th day after immunotherapy of DC-CIK (mean±square error)

Time	CD3 ⁺	CD3 ⁺ /CD4 ⁺	CD3 ⁺ /CD8 ⁺	CD4 ⁺ /CD8 ⁺	NK cell	NKT cell
Before treatment	47.46±9.66	29.12±9.44	30.81±9.58	0.99±0.23	16.46±3.57	6.53±1.19
After treatment	60.14±12.39	41.61±9.65	26.41±7.65	1.83±0.36	27.17±6.52	14.33±1.59

There is statistically significant difference before and after treatment, P<0.05.

Table 3: Clinical improvement before and after treatment before

Time	Fatigue or weakness	Lack of appetite	Partial Soreness	Insomnia and night sweat
Before treatment	10	9	8	6
After treatment	5	2	4	3

There is statistically significant difference before and after treatment, P<0.05.

(MHC) restrictive tumor killing feature of NK cells. CIK cells are featured by quick proliferation, free from the influence of immunosuppressor and being sensitive to multiple drug-resistant tumor cells. The research results showed that, the cellular phenotype experienced obvious changes; the ratios of total T lymphocyte (CD3⁺), T helper cells (CD3⁺/CD4⁺), NK cell (CD3⁺/CD16⁺56⁺) and NKT cell (CD3⁺/CD16⁺56⁺) obviously rose, while the ratio of T suppressor cell (CD3⁺/CD8⁺) declined significantly; the immunity functions remarkably improved; moreover, the QOL for patients was obviously improved and all of them tolerated the toxic and side effects. Immunotherapy of DC-CIK can improve their immune functions and the anti-tumor immune reactions. Besides, it also lays a sound basis on further treatment for those advanced breast cancer patients who have received radiotherapy and chemotherapy already.

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