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Cancer related fatigue and clinical outcome of dendritic cell vaccine in combination with cytokine-induced killer cell therapy in cancer patients

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Abstract

Cancer related fatigue significantly disrupts normal life for a portion of cancer survivors, and may persist for many years with cancer patients. Fatigue lasts longer than other treatment side-effects. There are increasing reports of using immunocyte therapy to treat cancer, and if it could be alternative relieve therapy in persons with cancer related fatigue is not so clear. Herein, we retrospectively evaluated the improvement of cancer related fatigue and safety of this therapy administered to cancer patients. 539 patients were treated with immunocyte therapy after conventional treatment.

Methods: The delayed-type hyper-sensitivity (DTH) skin test was used to measure the immune response. Investigated into cancer related fatigue, physical strength, appetite and sleep as clinical efficacy.

Results: The DTH skin tests showed that the positive cell-mediated cytotoxicity response rate was 82.7%. A large proportion of patients' cancer related fatigue (80%); appetite (94.1%), sleeping status (96.6%) and metal state (92.9%) were improved. Adverse reactions were fever (35.1%), insomnia (21.4%), anorexia (15.7%), arthralgia (8%), rash (3.3%). DC (dendritic cell) therapy combined with CIK (cytokine-induced killer cell) was observed no toxicity in patients.

Conclusion: This is the first time we reported combination of DC and CIK therapy to relieve the cancer related fatigue of patients after conventional therapy (surgery, radiotherapy, and chemotherapy). It may be an effective immunotherapy to reduce adverse reactions and a safety treatment. The data of CIK treatment time and CIK cell number provide strong support for further clinical treatment.

Keywords: Cancer related fatigue; Delayed-type hyper-sensitivity (DTH); Dendritic cell; Cytokine-induced killer cell

Introduction

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries [1]. As we know, surgery, radiotherapy and chemotherapy have different adverse effects; it cannot completely eliminate the tumor. The immunotherapy for cancer has been verified with improved clinical effect in various clinical trials [2]. This technology has become the fourth main treatment option for tumors, following surgery, radiotherapy and chemotherapy [3,4]. Adoptive immunotherapy based on DC and CIK cells has been successfully applied to treat solid cancers and also in hematologic malignancy tumor with certain efficacy [5,6]. In recent years, with the cancer related pain, vomiting and other symptoms get effective control. Cancer related fatigue significantly disrupts normal functioning and quality of life for a portion of cancer survivors, and may persist for many years with cancer patients. Cancer related fatigue has become an impact on the quality of life of patients with tumor. As a result of cancer related fatigue causes varied, there is no specific drug treatment. With some studies placing the percent of patients suffering from fatigue as high as 75-99%, Fatigue lasts longer than other treatment side-effects [7,8].

The occurrence and development of tumor are mainly due to the imbalance of defense system against the regulation of tumor, which leads to the failure of the competition between the body and the tumor. Therefore, it is able to mobilize the immune function to resist, and ultimately eliminate the cancer cells. At present, the common immune effector cells are cytokine induced killer (CIK) cells and dendritic cells (DC). Cytokine-induced killer (CIK) cells have become the preferred type of cells used in antitumor adoptive cell immunotherapy. CIK cells are heterogeneous cells that can be generated from human peripheral blood mononuclear cells (PBMCs) by culturing in the presence of a variety of cytokines *in vitro* [9].CIK cells have non-major histocompatibility complex (MHC)-restricted tumoricidal effect

[10,11]. CIK cells proliferate faster, but also has a T cell and NK cell antitumor dual function, the most important is their small cytotoxicity in normal cells, but it can be a lot of attack tumor cells [12,13]. Dendritic cell (DC) is the strongest professional antigen presenting cells (antigen presenting cells, APC), it can efficiently uptake, processing and presenting antigens. Immature DC has strong ability of migration, the mature DC can effectively activate naive T cells and initiation, regulation, and maintain a central role in the immune response [14].Taken together, human immune system is involved in recognition (DCs) and elimination (CIK) of malignant cells. Thus, the combination of DCs and CIKs results in considerable increase of immunity and shows more effective cytotoxic activity than single treatment [15,16]. DC-CIK cell therapies combined with chemotherapy have been reported beneficial in leukemia, renal tumor, liver cancer, and gastric cancer [17-21].

In recent years, there are many studies on the DC vaccine and CIK cells [22-25]. However, 1) Clinical cases is still limited and there are not enough data to confirm this benefit specifically for cancer related fatigue. 2) There is no clear consensus on how to optimize the CIK dosage, usage, scheme and safety and *in vivo* metabolic pathway dose not has final conclusion; the major adverse reaction is mild of DC vaccine, but in evaluation of DC vaccine side effects and its solutions need further study. DC and CIK cells kill tumor cells directly or indirectly and inhibit the proliferation of tumor cells by enhancing the anti-tumor immune response [26-28]. To address this issue, we investigated the delayed-type hyper-sensitivity (DTH) skin test and evaluate the subjective clinical outcome and safety of the regimen in 539 patients.

Subjects and Methods

Patients and trial design

The study was a single-institution, open-label, non-randomized, single-arm, clinical exploratory study performed at the Biological therapy and regenerative medicine transformation center, Lanzhou, China. Between June 28, 2013 and January 8, 2015. All patients in this study were patients with solid tumors and the histopathologic diagnosis was based on the World Health Organization criteria. The Research Ethics Committee of Hospital approved the ethical use of human subjects for this study, and was in accordance with the 'Treatment with Autologous Immune Cells (T cells and NK cel ls)' class III medical techniques policy of the Ministry of Health of China. All patients informed consent was obtained from each patient.

Generation of DCs and CIK cells

DCs were isolated from preparation Peripheral blood mononuclear cells (PBMC) by Ficoll-Paque (PAN Biotech, Aidenbach, Germany) density gradient centrifugation, seeded in 175 cm2 culture flasks at a density of 5 ×106 cells/ml for 2 hours at 37°C in AIM-V medium (Invitrogen). After 2 hours, the non-adherent cells were removed for culturing CIK while adherent mono-cytes were cultured for DCs in AIM-V medium (Invitrogen) (autologous plasm) supplemented with 1000 units/ml granulocyte macrophage colony-stimulating factor (GM-CSF; Novartis, New Jersey, USA) and interleukin-4 (IL-4) 500 U/ml (Strathmann Biotech, Hannover, Germany) for 7 days. On day 4, DCs maturation could be achieved by adding 100 ng/ml tumor necrosis factor-α (TNF-α) (R&D Systems, Minneapolis, MN, USA); On day 6, DCs were pulsed with specific antigens. On day 7, DCs were harvested, washed, and suspended again to a final concentration of 1.0 × 107 cells in saline solution for injection (Figure 1).

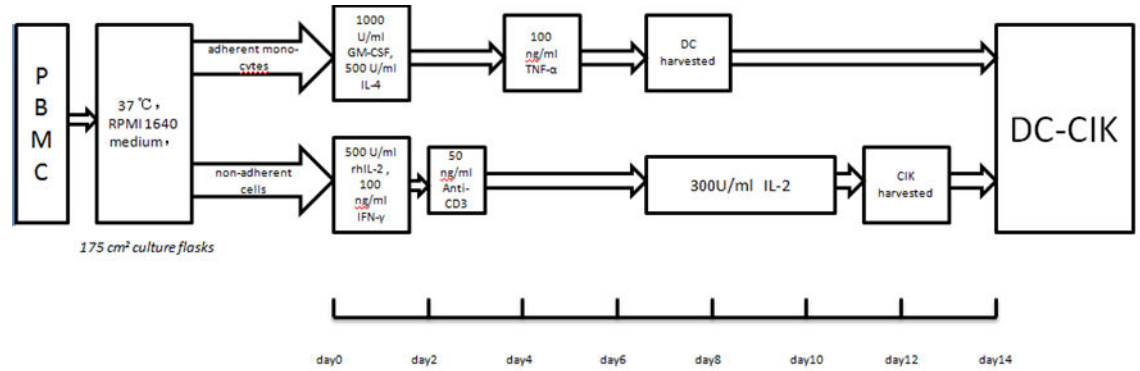

Figure 1: Manufacturing process of CIK and DC cell agent.

Generation of CIK: Non-adherent cells were prepared in CIK medium (AIM-V medium containing 500 U/ml rhIL-2 and 100 ng/ml IFN-γ (Hofman La Roche), 50 ng/ml Anti-CD3 (e-Bioscience) at the density (3-5) × 10^6/ml, then were seeded in 2 L culture bag. Cells were incubated in a humidified atmosphere of 5% CO2 at 37°C.CIK medium was changed every 2 or 3s,. CIK cells were harvested on day 11 and 13 (Figure 1).

Observation indicators: The phenotype of the cultured cells was analyzed by flow cytometry. The immune phenotypes of CD80+, CD83+, CD86 and HLA-DR+ for the DCs (Figure 2,3), the percentage of CD3+CD56+cells of the CIK cells were not less than 10%. Flow cytometry was performed using an FC500 (Beckman-Coulter), and CXP analysis software (Beckman-Coulter) was used for analysis. The number of CIK cells per time of the transfusion should be no less than

1×10^{10}, the number of DC cells per time of the transfusion should be no less than 1×10^{7}.

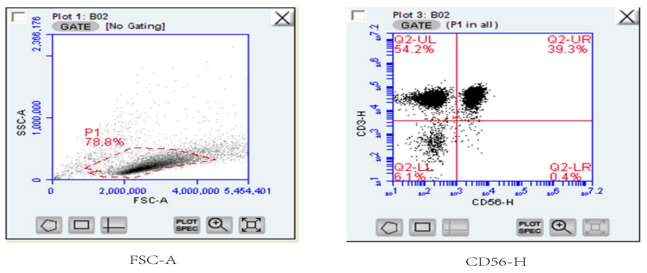

Figure 2: The immune phenotypes of the CIK cells.

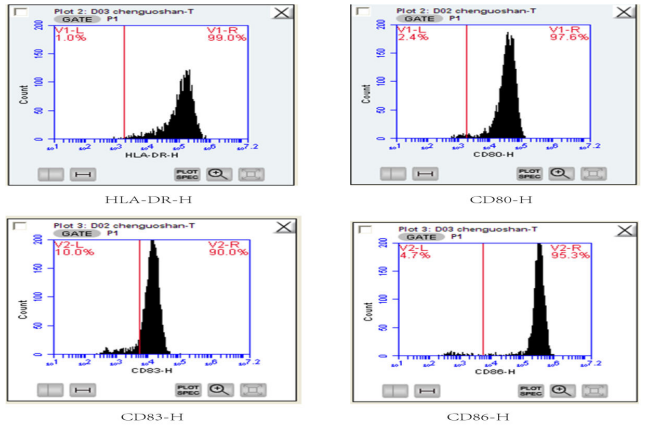

Figure 3: The immune phenotypes of CD80+, CD83+, CD86 and HLA-DR+ for the DCs.

Therapeutic methods

After a week of blood collection, 6×10^{7} cells DCs were administered at three times, of which 1×10^{7} cells were injected intravenously, and the other 1×10^{7} cells were injected into the body by subcutaneous injection. CIK cells total input 1×10^{10} cells. For the first time: the number of CIK cells should be 1×10^{9}; the second time: the number of CIK cells should be 2×10^{9}; The third return the number of CIK cells lose is 3.0×10^{9}. The fourth time to CIK cells number were 4.0×10^{9}. The subcutaneous injection sites were the ventromedial regions of the upper arms and thighs close to the regional lymph nodes and were rotated clockwise.

Immune response

The DTH skin test was used as the index of the immune response of the DC vaccine in combination with CIK therapy in the patients with cancer. Tumor lysate (40 μg/0.1 ml) was administrated intradermally into the forearm of each patient one week after the end of therapy. The results of the DTH test were defined as markedly positive >10 mm diameter of erythema; positive, 5-10 mm; weakly positive, 2-5 mm; and negative, <2 mm after 48 h.

Results

Patients

Between June 8, 2008, and January 8, 2015, there were 539 cancer patients (316 males and 223 females) were included in the present study. The mean age was 57.4 (range, 17-86) years. The primary tumors were the lung cancer (18.5%), Gastric cancer (13.8%), colorectal cancer (11.2%), HCC (10.0%), breast cancer (9.0%) and other regions (37.5%) (Table 1). Among the 539 cases, 109 (20.22%) had surgery and immunotherapy, 52 (9.65%) had only immunotherapy, a large proportion of patients had combined conventional ways (radiotherapy or chemotherapy, surgery) with immunotherapy (Table 2).

Characteristics	Value	%
Age(years)		
Range	17-86	
Mean ± SD(range)	57.4 ±11.7	
Gender		
Male	316	58.7
Female	223	41.3
Tumor type		
Lung cancer	100	18.5
Breast cancer	49	9.0
HCC	54	10.0
Gastric cancer	74	13.8
Colorectal cancer	60	11.2
Others	202	37.5

Table 1: Disease characteristics of patients.

Pattern	Number	Percentage
immunocyte therapy	52	9.65%
Surgeryimmunocyte therapy	109	20.22%
Chemotherapy, radiotherapyimmunocyte therapy	295	54.73%
SurgeryChemotherapy, radiotherapyimmunocyte therapy	83	15.40%

Table 2: The method of treatment for patients.

Efficacy

QOL: QOL was recorded as an improvement in the cancer of patients, Out of these 539 patients, 501 (92.9%) show edapositive improvement in their mental state, 507 (94.1%) had improved appetite, 521 (96.6%) were able to sleep better. Cancer related fatigue had significant improvement in patients (80%, 388/485) by Piper fatigue questionnaire (Table 3). In this study of 539 cancer patients, 10% (54) had no significant cancer related fatigue before the biological treatment.

Characteristics	Improvement in general condition		
	Significant (%)	Slight (%)	No change or worse (%)
Fatigue*(485)	155 (32)	233 (48)	97 (20)
Appetite(539)	460 (85.3)	47 (8.7)	32 (5.9)
Sleeping status (539)	447 (82.9)	74 (13.7)	18 (3.4)
Metal state (539)	258 (47.9)	243 (45.0)	38 (7.1)

Table 3: Subjective clinical outcomes following the use of the DC vaccine + CIK therapy. DC: dendritic cell; CIK: cytokine-induced killer. Fatigue*(485): In this study of 539 cancer patients, 10% (54)had no significant fatigue before the biological treatment.

DTH: DTH as an indicator of immune response can serve as an efficacy end point for DC cells and CIK cells immunotherapy. The total number of patients was 539, 54 patients (10.0%) had a strongly positive response, 130 patients (24.1%) had a positive response, and 262 patients (48.6%) had a weakly positive response. In total, 82.7% of patients (446 of 539) had a positive immune response and the other 17.3% of patients (93 of 539) failed to show an immune response (Table 4).

Results of DTH	Definition (mm)	No (%)
Markedly positive	>10	5410
Positive	5-10	13024.1
Weakly positive	2-5	26248.6
Negative	<2	9317.3

Table 4: DTH skin test following the use of the DC vaccine in combination with CIK therapy. DTH: delayed-type hypersensitivity; DC: dendritic cell; CIK: cytokine-induced killer.

Adverse Effects: Adverse effects were assessed in 539 patients in this study.189 (35.1%) developed fever, 115 (21.4%) developed insomnia, 85 (15.7%) developed anorexia, 43 (8%) developed joint soreness, and 18(3.3%) developed skin rash. No toxicity resulted from DC vaccine and CIK cell therapy (Table 5).

Characteristics	No (%)
Fever	35.1%
insomnia	21.4%
anorexia	15.7%
sore joints	8%
skin rash	3.3%

Table 5: Side-effects following the use of the DC vaccine in combination with CIK therapy. A patient may have had more than one side-effect.

Discussion

Immunotherapy is able to mobilize the immune function to resist, and ultimately eliminate the cancer cells. It is to enhance anti-tumor immunity by stimulating and mobilizing its own immune system, and to control and kill tumor cells by human intervention. Immunotherapy is a promising treatment option, and is considered to be the fourth cancer treatment [29,30]. At present, the common immune effector cells are cytokine induced killer (CIK) cells and dendritic cells (DC). Although in recent years on the DC vaccine and CIK cells of many studies. On the one hand, clinical cases is still limited and there are no clear consensus on how to optimize the CIK dosage, usage, scheme and safety; On the other hand: The problem of combination of DC and CIK to relieve the cancer related fatigue of patients after surgery, radiotherapy, and chemotherapy is unknown.

Previous studies suggest that DC and CIK therapy as adjuvant therapy found positive cell-mediated cytotoxicity response rate of 76.9% was detected in 121 patients by the DTH skin tests [31]. However, the sample size is small. In our center, patients who received an adjuvant immunotherapy using the DC and CIK cell agent after conventional treatment for cancer had 82.7% in 539 patients were positive for the DTH skin-test at 48 h post-treatment. The effect of the CIK cell agent and DC on DTH and Subjective clinical outcome statistically significant after adjustment for a portion of cancer survivors. In addition, our study showed that for cancer patients who had significant increase of cancer fatigue before the biological treatment, sequential DC-CIK cell therapy, 80% (388/485) cases exhibited improvement in cancer related fatigue.

About DC and CIK *in vivo* effect duration, there is no objective indicator to reflect. But the quality of life has become the most important indicator of the evaluation of cancer treatment, so the results of the study showed that the cell treatment before and after the comparison, followed up for 3 months after the treatment of patients with fatigue status of the improvement of the situation. Lee JH et al. showed that they were scheduled to receive the CIK cell agent 16 times (4 treatments at a frequency of once per week, followed by 4 treatments every 2 weeks, then 4 treatments every 4 weeks, and finally 4 treatments every 8 weeks) [32].

Cancer related fatigue frequently worsens during treatment and is recognized as a factor limiting patient adherence to cancer therapy [33]. A large part of the patients are willing to continue to do, the main reason is because it can change the cancer related fatigue by biotherapy. Previous studies using DC and CIK cells showed significant benefits in improving physical strength [31], but there are no statistical in cancer related fatigue. Our results are in agreement with those reported studies, and in our center, cancer related fatigue had significant improvement in patients (80%, 388/485). In recent years, with the cancer related pain, vomiting and other symptoms get effective control of cancer related fatigue has become an impact on the quality of life of patients with spleen tumor. And cancer patients with persistent fatigue reasons not entirely clear, there is evidence that including chronic inflammation, plant nerve disorders and other reasons may contribute to the failure in fatigue in normal neuronal function and effect. Chronic inflammation is likely to be the level of the immune system is relatively low, and a variety of functional defects which may cause the patient's own immune system does not effectively remove inflammation.

Cancer-related fatigue is a non-specific, highly subjective multidimensional experience, this feeling cannot be tested directly

observer, increasing the difficulty of clinical research. the cause of cancer-related fatigue study is still in the exploratory stage, is generally believed that cancer-related fatigue incentives are the following aspects: the tumor itself factors, tumor treatment factors (including surgery, chemotherapy, radiotherapy, drug treatment), complications (anemia, infections, malnutrition.) caused by tumors or cancer treatment, chronic symptoms (pain, irregular sleep, low immunity), emotional factors (anxiety, depression) [34].Patients in the DC-CIK therapy experienced fewer adverse side effects and associated with less fatigue and better appetite. This could be due to an improved immune response in these patients. Many studies have confirmed the CIK as adjuvant therapy can reduce the tumor burden in patients with advanced cancer [35]. Lung cancer patients after surgery to reduce the load, the more immune cells to attack tumor cells, and it can help identify and eliminate micrometastases [36]. Our results show that DC / CIK therapy can improve CRF, according to our experience, increasing the input cell dose and the frequency of patients entered more than four times, the effect will be better.

Changes in the size of the tumor by MRI (Magnetic Resonance Imaging) or CT (Computed Tomography) as an important indicator of cancer treatment, but it is difficult to accurately evaluate the clinical results of the entire anti-tumor therapy. In order to evaluate the clinical outcome, the effect of other combined therapies, such as surgery, radiotherapy or chemotherapy, should be excluded, but it is impossible performed in cancer patients who are restricted to receiving only the regimen of DC vaccination in combination with CIK therapy. This study attempts to use the immune response to reflect the clinical effect, to demonstrate the treatment of DC and CIK of the therapeutic effect [31]. Studies have shown that surgery, radiotherapy and chemotherapy can cure some tumors, not due to killing all the cancer cells, but when the tumor burden significantly reduced immune function recovery, cleared the minimal residual tumor or residual tumor cells significantly inhibited proliferation, indicating that immune tumor growth is important. In the preliminary clinical results, it can be used as an ideal adjuvant therapy because of the antigen specificity of the immune system and the safety of the tumor vaccine. This may be the biggest reason for many cancer patients to continue to receive DC-CIK treatment to improve the quality of life of patients.

In conclusion, our results demonstrate the effectiveness of biological immune for cancer related fatigue. These results may provide an evidence for DC and CIK treatment, and suggest that the combination DC and CIK may be a safe strategy for patients with cancer to reduce side reaction and relieve fatigue.

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Author Disclosure Statement

The authors declare that no conflicting financial interests exist.

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