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Original article

Effects of cytokine-induced killer cell treatment in colorectal cancer patients: A retrospective study

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ABSTRACT

Cytokine-induced killer (CIK) cells are ex vivo generated heterogeneous NK-like T-lymphocytes, which have anti-tumor effects in vitro and in vivo. This present study was conducted to evaluate the effects of autologous CIK cell immunotherapy on the prognosis of colorectal cancer patients. Progression-free survival (PFS), overall survival (OS) and immune cells were assessed. We found that the percentages of $CD8^+$, $CD3^+$ $CD56^+$, $CD3^-$ $CD56^+$ cell subsets were significantly increased from 19.7 ± 6.3%, 13.8 ± 7.9%, 1.0 ± 1.2% to 35.8 ± 11.6% ($P < 0.001$), 20.9 ± 12.5 ($P < 0.001$), 14.4 ± 9.5% ($P < 0.001$), respectively in the CIK group after 14 days of incubation. The median PFS and median OS in the CIK group were 25.8 months and 41.3 months respectively, while 12.0 months and 30.8 months in the control group. The PFS and OS curves of the CIK group and control group indicated that there were also statistically differences between two groups in PFS (log-rank, $P = 0.01$) and OS (log-rank, $P = 0.037$). Our results indicate that CIK cell immunotherapy in combination with chemotherapy can reduce the recurrence rate and promote the survival time of patients with colorectal cancer.

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1. Introduction

Colorectal cancer is the third most commonly diagnosed cancer in males and the second in females and the fourth leading cause of cancer-related death worldwide. More than 1.2 million of new colorectal cancer cases were diagnosed globally every year and 608,700 deaths estimated to have occurred in 2008 [1]. The incidence of colorectal cancer is different throughout the world. In United States, both incidence rate and mortality rate of colorectal cancer rank third among all cancers in both men and women, which indicate that colorectal cancer is becoming an important public health problem in developed countries [2]. It is unfortunate that surgery, chemotherapy, radiotherapy and hyperthermia usually fail to eradicate tumor lesions completely and cause many adverse events [3]. Therefore, an effective therapy is needed to help eradicate tumors lesions and prolong the survival of patients with colorectal carcinoma.

In recent years, immunotherapy has become one of the multidisciplinary treatments for a variety of malignant tumors and shown encouraging efficacy and minimal adverse events in cancer therapy [4]. Studies have shown that some immune cells, such as NK-like T-lymphocytes (CIK) cells, lymphokine-activated killer cells, natural killer (NK) cells, and dendritic cells, exert immunoregulatory effects on various human cancer cells [5,6]. CIK cells are a subset of T-lymphocytes which express both the CD3 and the CD56 markers, non-major histocompatibility complex (MHC)-restricted. They are generated ex vivo by incubation of peripheral blood mononuclear cells (PBMCs) with interferon-gamma (IFN-γ) followed by anti-CD3 monoclonal antibody (mAb) and interleukin-2 (IL-2) [7]. They represent high level of anti-tumor cytotoxicity in vitro and in vivo and may have enhanced proliferative and cytotoxic activity compared with lymphokine-activated killer (LAK) cells and tumor-infiltrating lymphocyte (TIL) cells [8,9]. To evaluate the clinical outcomes of CIK cell immunotherapy, we conducted a randomized clinical trial with autologous CIK cells in colorectal cancer patients.

2. Materials and methods

2.1. Patients

This study included 60 patients with histologically or clinically confirmed colorectal cancer admitted to The First Affiliated Hospital of Nanjing Medical University from December 31, 2005

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to May 1, 2012. The 60 patients with colorectal cancer were divided into two groups: thirty patients who received chemotherapy and autologous CIK cell immunotherapy were defined as the "CIK group". The other thirty patients of the corresponding period who received chemotherapy alone were defined as the "control group". Patients having an expected survival duration of more than 3 months, a Karnofsky performance status score higher than 60, no other malignant cancer history were included in this study. While with ischemic heart disease, cerebrovascular disease, chronic airway obstruction disease and other chronic diseases were excluded. All the eligible patients were followed up every 2 months from the date of initial treatment to December 31, 2013, or to the time of death by telephone calls. Clinical information was performed by oncology specialists, including a complete blood count and chest/abdominal/pelvic computed tomography scan according to the NCCN guideline. The research protocol was approved by the institutional review board of Nanjing Medical University.

2.2. Treatment

All patients in the CIK group and the control group received 6 cycles of multidrug adjuvant chemotherapy based on 5-FU (FOLFOX or XELOX) before CIK cell immunotherapy. The patients in the immunotherapy group were given autologous CIK cell treatment at a 1-month interval after adjuvant chemotherapy. For each patients of immunotherapy group, 5 mL of venous blood would be obtained to analyse CIK phenotypes by flow cytometry before and after CIK cell transfusion. The patients in the control group did not receive CIK cell immunotherapy after 6 cycles of chemotherapy.

2.3. Preparation and transfusion of CIK Cells

First, 5 mL peripheral venous blood was collected from each patient of immunotherapy group in the morning under fasting conditions, which was analyzed for CIK phenotypes by flow cytometry. Blood cells were preserved and incubated with fluorescein isothiocyanate (FITC)-conjugated anti-CD4, cyanine-5 (CY5)-conjugated anti-CD3 and phycoerythrin (PE)-conjugated anti-CD8/CD56 for 30 min. The temperature was maintained at 4 °C. The flow cytometry analysis was performed immediately after washing three times and resuspended with PBS.

Then, 50 mL of heparinized peripheral blood was obtained from each patient by flow cytometry. Peripheral blood mononuclear cells (PBMCs) were collected by CS-3000 Plus blood cell separator and isolated by Ficoll-Conray density gradient centrifugation. Cell density was about 5×10^8–1×10^9 ml^{-1}, and the cells were incubated in fresh serum-free AIM-V medium and humidified atmosphere of 5% CO_2. The temperature was maintained at 37 °C. On the initial day, RhIFN-γ (2000 U/mL) was added. Anti-CD3 mAb (50 ng/mL) and rhIL-2 (1000 U/mL) were added after incubation for 24 h. The cell suspensions were maintained in subculture with fresh medium with 1000 U/mL rhIL-2. All of the cells were washed and refilled every 2–3 days for 3–4 weeks. Cell phenotypes were identified on days 0, 7, 10, 14 and 21 by flow cytometry respectively. The CIK cells were washed 3 times with normal saline and resuspended in normal saline with human serum albumin before CIK cell infusion. The final cell products were checked twice for possible contamination of bacteria, fungi and endotoxins and assessed by the dye-exclusion test. The viable cells must account for more than 95%. The eligible CIK cells were collected, washed and transfused back into patients by intravenous injection. The main procedure for ex vivo expansion of CIK cells is shown in Fig. 1.

2.4. Statistical analysis

The diversifications of T-cell subtype distributions before and after CIK cell transfusion were expressed as means ± SE and were tested by the *t*-test. Differences in distribution of demographic and clinical characteristics between the immunotherapy and control groups were evaluated using the χ^2 test. The OS curves and PFS curves were calculated using the Kaplan–Meier method. OS was defined as the time elapsed from the date of surgery to either the date of death or the date of last contact. PFS was defined as the time calculated from the date of surgery to the first progression or the date of last follow-up information. All analysis was carried out with SPSS software (version 19.0, for windows), using two-sided *P*-values. *P*-values of < 0.05 were considered statistically significant.

3. Results

3.1. The characteristics of patients

The characteristics of patients are summarized in Table 1. In our study, a total of 60 patients were included in this study. Among these patients, 56 patients had received surgical resection while the other 4 patients had not received surgical resection for widespread metastases. Only two patients accepted radiotherapy. No statistical difference was found in demographic and clinical characteristics between these two groups. Among these patients of immunotherapy group, eleven patients accepted only 1 cycle of CIK cell transfusion, eight patients received 2 cycles, six accepted 3 cycles and five accepted 4 cycles. The Karnofsky Performance scores of all these patients were over 60.

3.2. Phenotypic analysis of CIK cells

The T-lymphocytes subsets of the patients in immunotherapy group were evaluated by flow cytometry in peripheral blood before and after immunotherapy (Fig. 2). All CIK cells were administered according to the following criteria: the percentages of $CD3^+$ and $CD8^+$ cells must exceeded 70% and 40%, respectively, and $CD3^+$ $CD56^+$ cells were more than 30%. The final cell products were assessed for viability by the dye-exclusion test and the proportions of viable CIK cells exceeded 95%. Then, cell cultures would be evaluated for possible contamination of bacteria, fungi and endotoxins by the Department of Microbiology and our laboratory to make sure that they were safe to patients. Before CIK cell transfusion and after 10 days CIK cell transfusion, PBMCs were obtained from the same patient and phenotypic analysis of CIK cells changes were determined by flow cytometry. The percentages of $CD8^+$, $CD3^+$ $CD56^+$, $CD3^-$ $CD56^+$ cell subsets were significantly increased from 19.7 ± 6.3%, 13.8 ± 7.9%, 1.0 ± 1.2% to 35.8 ± 11.6% ($P < 0.001$), 20.9 ± 12.5 ($P < 0.001$), 14.4 ± 9.5% ($P < 0.001$) (Fig. 2), respectively except for $CD3^+$(from 65.6 ± 10.0% to 69.7 ± 13.9%, $P = 0.07$), whereas the percentages of $CD4^+$ cells were significantly decreased from 38.0 ± 9.9% to 25.4 ± 10.9% ($P < 0.001$) (Fig. 3, Table 2).

3.3. Prognosis

We evaluated the effects of CIK cell treatment as an adjuvant therapy on the prognosis of colorectal carcinoma in patients undergoing chemotherapy after surgery or without surgery by PFS and OS. During the time of the follow-up, there were 5 fatalities in the CIK group while there were 10 fatalities in the control group. The median PFS and OS in the CIK cell treatment group were 25.8 months and 41.3 months, respectively, which was improved compared with that in the control group (12 months and

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