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Chemotherapy in combination with cytokine-induced killer cell transfusion: An effective therapeutic option for patients with extensive stage small cell lung cancer

Jianmin Huang [a], Quancheng Kan [b,*], Lan [a], Xuan Zhao [a], Zhen Zhang [a], Shuangning Yang [a], Hong Li [a], Liping Wang [d], Li Xu [d], Zhe Cheng [e], Yi Zhang [a,c,f,*]

[a] Biotherapy Center of the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China
[b] Department of Pharmacology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China
[c] School of Life Sciences, Zhengzhou University, Zhengzhou, Henan, China
[d] Department of Oncology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China
[e] Department of Pneumology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China
[f] Engineering Key Laboratory for Cell Therapy of Henan Province, Zhengzhou, Henan, China.

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ABSTRACT

Background and aims: In the past decade of clinical studies, the combination of chemotherapy with cytokine induced killer (CIK) cell transfusion has confirmed a promised efficacy in several types of cancer. CIK cells are a mixture of T lymphocytes, generated from peripheral blood mononuclear cells induced by multiple cytokines. This study was aimed to evaluate the clinical efficacy of chemotherapy combined with CIK- cell therapy in patients with extensive stage small cell lung cancer (ES SCLC). **Patients and Methods**: Forty four patients with ES SCLC were enrolled in this study. All the patients received treatment from Oct 2010 to Sep 2013 in the First Affiliated Hospital of Zhengzhou University. Included patients were equally divided into 2 groups according to the treatment strategies. Patients in the combined treatment group received chemotherapy combined with CIK-cell transfusion and patients in the control group received chemotherapy alone. The short-term effects, overall survival (OS), progress free survival (PFS) and therapy-related adverse events were analyzed retrospectively. **Results**: Short-term efficacy evaluation indicated that the total response rates in the combined treatment group and control group were 40.9% (9/22) and 9.1% (2/22), respectively. There was a significant difference between the two groups ($p = 0.0339$). Furthermore, the PFS of the combined treatment group was significantly longer than that of the control group (8 *vs.* 4 months, $P = 0.005$). No severe side effect was observed after transfusion of CIK cells. **Conclusion:** These results indicated that chemotherapy combined with CIK-cell immunotherapy might provide a safe and effective treatment for patients with ES SCLC.

Introduction

Lung cancer is the most common cancer worldwide, and it is often diagnosed at the advanced stage. Small cell lung cancer (SCLC) accounts for approximately 13% of all patients diagnosed as lung cancer [1], and about two-thirds of patients presenting with extensive stage disease at the time of diagnosis [2]. Concurrent chemoradiotherapy is highly recommended in guidelines for limited stage SCLC (LS SCLC) patients [3–5], while chemotherapy alone is the standard treatment for patients with extensive stage small cell lung cancer (ES SCLC) [3–5]. Although the patients with ES SCLC have a high response rate to the first-line chemotherapy, the response is short and the recurrence occurs frequently [2,6]. From the time of diagnosis, the reported median survival range of ES SCLC is only 8–13 months [7] and the 2-year survival rate is 5.2–19.5% [8–10]. Therefore, developing an effective therapy is important in order to improve the prognosis of ES SCLC.

Adoptive immunotherapy, which eliminates cancer cells through the transfusion of generated immune cells *in vitro*, is proving to be an effective strategy for cancer therapy [11]. Adoptive immunotherapy with cytokine-induced killer (CIK) cell transfusion remains a promising adjuvant treatment for many malignant tumors [11]. CIK Cells are a mixture of T lymphocytes, comprising $CD3^+CD56^+$ cells, $CD3^-/CD56^+$ natural killer (NK) cells, and $CD3^+/CD56^-$ cytotoxic T cells [12]. Among them, $CD3^+CD56^+$ T cells are the main effector cells [13]. Because of their non–MHC restricted tumor killing activity, CIK cells have a broad antitumor spectrum with powerful *anti*-neoplasm effect. However, accumulated data suggested that immunotherapeutic and conventional

* Corresponding authors at: No. 1 Jianshe Road, Zhengzhou, 450052, Henan, China.
E-mail addresses: kqc2008@163.com (Q. Kan), yizhang@zzu.edu.cn (Y. Zhang).

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strategies alone are often ineffective to eradicate big tumors or metastasis. To improve the prognosis of advanced cancer patients, conventional cancer treatment combined with immunotherapy might be an essential strategy [14–16]. The combination of CIK cells with other modalities had demonstrated a therapeutic promise in patients with some solid malignant tumors [17–25]. However, there are few reports for its application in patients with ES SCLC.

In this study, the patients with ES SCLC were treated with chemotherapy combined with CIK-cell transfusion or chemotherapy alone. The safety and efficacy of the combined therapy were evaluated.

Patients and methods

Patients.

The medical histories of patients with ES SCLC were reviewed from a computerized database in the First Affiliated Hospital of Zhengzhou University from Oct 2010 to Oct 2013. The patient eligibility and selection criteria were as follows:(1) Having cytology or histology confirmed SCLC and clinical evidence of extensive stage disease (defined by distant metastasis, contra-lateral hilar-node metastasis, or malignant pleural effusion). (2) Adequate hepatic and renal function. (3) Aged 18 or older with Karnofsky Performance Status of 70–100. (4) The bone marrow functioned normally (white blood cells $>4.0 \times 10^9$ /L, lymphocyte cells $>0.8 \times 10^9$ /L, blood platelet $>100 \times 10^9$/L). Exclusion criteria for the patients included: (1) Preexisting autoimmune disorder. (2) With immunodeficiency condition. (3) Concomitant serious ongoing infection. (4) Being pregnancy or lactating. Forty-four patients were included in this study for further analysis. Among them, 22 patients who received 6 cycles of chemotherapy combined with CIK-cell transfusion were divided into the combined treatment group, and the other 22 patients who received 6 cycles of chemotherapy only were used as control group for comparisons.

Preparation for CIK cells.

CIK cells were cultured as described in previous reports [26]. In summary, the peripheral blood was collected from the patients in the combined treatment group. The peripheral blood mononuclear cells (PBMCs) were isolated by ficoll density gradient (Tianjin, HY, China) centrifugation. The PBMCs were then cultured in fresh serum-free medium (Takara, Japan) with 1000 U/mL IFN-γ (Shanghai KL, China), at 37 °C with 5% CO_2 for 18–24 h, then 1000 U/mL recombinant human IL-2 (rhIL-2, Beijing SL, China) and 100 ng/mL CD3 monoclonal antibody (OKT3, Boehringer Mannheim, Germany) were added to the medium. The cells were fed with fresh complete culture medium containing rhIL-2 and 1–2% autologous serum every 2–3 days until day 14. The CIK cells were confirmed free of bacteria, fungus contamination before transfusion.

Phenotypic analysis.

Phenotypic analysis and detection of the inhibitory molecular expression on CIK and the PBMCs, were performed by using fluorescence labeled monoclonal antibodies to CD3, CD56, CD16, CD4, CD8, PD-1, PD-L1, Tim-3, LAG-3 and TIGIT (BD Pharmingen, USA). Then the stained cell population was analyzed by flow cytometry (BD Canto II, USA).

Cytokine production analysis.

CIK cells from 5 patients were chosen for the cytokine production detection. The method was as described in previous report [22]. In briefly, CIK cells were stimulated with phorbol myfismte acetate (PMA, 1 μg / mL, Sigma, USA), ionomycin (1 μg /mL, Sigma, USA) and brefeldin A solution (1 × BFA, Biolegend, USA) for 5 h at 37 °C in 5% CO_2. Then cells were harvested and stained with antibodies against CD3, CD4, CD8 and CD56 for 30 min on ice in the dark followed by 4% formalin fixation for 20 min. Subsequently, after being washed with permeabilization washing buffer (Biolegend, USA), cells were stained with antibodies against IFN-γ, Perforin and Granzyme-B for 20 min. The cytokines production of the CIK cells was detected by flow cytometry (BD Canto II, USA).

CD107a degranulation assay.

For the degranulation assay, as described by previously report [22, 26], CIK cells (2×10^6) were stimulated for 5 h in complete medium with 1×10^5 human SCLC cell line H1688 (purchased from Cellbank of Chinese Academy of Sciences). BFA and *anti*-CD 107a monoclonal antibody (mAb) were also added. PMA and Ionomycin were added in the positive group and CIK cells alone were used as negative control. After 5 h of stimulation, cells were washed and then labeled with anti-CD3, anti-CD56 and anti-CD107a mAbs for 15 min at 4 °C. Cells were analyzed on the FACSDiva™ software.

Cytotoxicity assay.

To determine the cytolytic activity of CIK cells against tumor cells, flow cytometry analysis was performed as previously reported [26]. In brief, 5×10^6 cells /mL SCLC cell line H1688 was labeled with 5 nM CFSE (Invitrogen) for 10 min. Then CIK cells were added to CFSE-labeled H1688 cells at different effector to target ratios for 5 h. CFSE labeled H1688 cell line, in the absence of CIK cells, were used as a control to assess spontaneous cell death. Then, the propidiumiodide (PI) permeability of target cells was analyzed by flow cytometry. The percentage of lysis rate was calculated using the following formula: experimental mortality − spontaneous mortality/100 − spontaneous mortality × 100%.

Treatment.

CIK-cell transfusion The procedure of Chemotherapy combined with CIK-cell immunotherapy was performed in accordance with the "Treatment with Autologous Immune Cells (T cells, NK cells)" class III medical techniques policy of the Ministry of Health of China. Informed consent was obtained from all the included patients before treatment initiation.

For patients in the combined treatment group, blood collection for CIK cells preparation was performed on day 0 prior to initiating chemotherapy, and the CIK cells were transfused on day 14. One course of chemotherapy plus CIK-cells consisted of 1 circle of chemotherapy treatment followed by 1 transfusion of CIK cells, and a total of 6 courses of chemotherapy plus CIK-cells were given to each patient (Fig. 1).

Chemotherapeutic treatments 6 cycles of chemotherapy were administered to the combined treatment group and the control group. The chemotherapy regimens had no significantly difference in two groups (Table 2).

Evaluation of clinical efficacy.

In accordance with the Response Evaluation Criteria in Solid Tumors (RECIST), the short-term clinical efficacy was evaluated as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). **Follow-up and data collection.** Follow-up for the cases was started 4 weeks after the first circle of chemotherapy in the combined treatment group and the control group. Telephone follow-up was performed every 4 weeks at the first 3 months and thereafter every 3 months. The deadline for the follow-up was up to 31 December 2013. The necessary hematologic examinations were carried out at each visit, and computed tomography scans for chest and upper abdominal (plain + enhanced scan), magnetic resonance imaging scans for brain + neck (plain + enhanced imaging) and bone scan were performed when tumor recurrence or metastases were suspected. The results of hematologic examination and imaging studies were collected from the electronic medical records at the First Affiliated Hospital of Zhengzhou University. The overall survival (OS) of patients was calculated from the first time of the first circle of chemotherapy to the time of patient death or the follow-up deadline. The progression-free survival (PFS) was calculated from the first time of the first circle of chemotherapy to the time of the first visible tumor progression observed by imaging studies.

Statistics

The data were expressed as mean, median, and ranges. *t*-test, one-way NOAVA and χ^2 test were used to analyze parameters for statistical