Effects of DC−CIK cell immunotherapy combined with chemotherapy on immune function, coagulation function and tumor stem cell markers in patients with recurrent ovarian cancer

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ABSTRACT

Objective: To investigate the effects of immunotherapy with dendritic cells (DC)-cytokine induced killer cells (CIK) combined with chemotherapy on immune function, coagulation function and tumor stem cell markers in patients with recurrent ovarian cancer. Methods: A total of 80 cases in our hospital for treatment of recurrent ovarian cancer patients were selected as the research object, they were divided into chemotherapy group (n=35) and combined treatment group (n=45) according to whether or not to receive cellular immunotherapy, chemotherapy group received TC chemotherapy, combined therapy group were given DC-CIK cell immunotherapy combined with TC chemotherapy group, the two groups were treated with 21 d for a cycle, 3 cycles were treated; The changes of immune function, coagulation function and tumor stem cell markers were compared between the day of blood collection and the end of treatment for 7 d. Results: After the end of treatment for 7 d, the CD3+, CD3+CD4+, CD4+/CD8+, NK cell number of combined treatment group were significantly higher than that of blood collection day, the number of CD4+ CD25+ was significantly lower than that of blood collection day, The improvement of peripheral blood T lymphocyte subsets in the combined treatment group was better than that in the chemotherapy group; After the end of treatment for 7 d, the FIB levels of two groups were significantly decreased than those of the blood collection day, the difference was statistically significant; After the end of treatment for 7 d, the CD133 and DDX4 levels of two groups were significantly lower than that of the blood collection day, and the combined treatment group was significantly lower than the chemotherapy group, the difference were statistically significant. Results: DC-CIK cell immunotherapy combined with chemotherapy can significantly improve the immunity and the level of FIB of patients with recurrent ovarian cancer, and regulating the level of serum tumor stem cell markers in patients, it has positive significance to improve the prognosis of patients, and is worthy of popularization and application in clinic.

1. Introduction

Ovarian cancer mortality ranks first among women with malignant reproductive tumors. The recurrence rate after 2-3 years is more than 70%, which is one of the important causes of death in patients with ovarian cancer[1]. How to prolong the survival time of patients and improve the quality of life is the primary problem in the treatment of recurrent ovarian cancer. Immune cell combination therapy combined with chemotherapy is a new anti-tumor treatment. The treatment has been applied to a variety of malignant tumors, but few reports used in recurrent ovarian cancer[2]. In this study, cytokine induced killer (CIK)-dendritic cell (DC) immunotherapy was used in the treatment of recurrent ovarian cancer, and observe the influence of immune function, coagulation function and tumor stem cell marker level on the patients. It provides a theoretical basis for exploring a more effective treatment regimen for recurrent ovarian cancer.

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2 Data and methods

2.1. General information

A total of 80 patients with recurrent ovarian cancer who were treated in our hospital from January 2016 to January 2017 were selected as the subjects. The patients were divided into chemotherapy group and combined treatment group according to whether they received cellular immunotherapy. There were 35 patients in chemotherapy group, aged 34-76 years old. Pathological types: serous adenocarcinoma in 23 cases, mucinous adenocarcinoma in 5 cases, endometrioid carcinoma in 3 cases, clear cell carcinoma in 4 cases. Pathological grading: G1 in 3 cases, G2 in 5 cases, G3 in 27 cases. Lymph node invasion: 12 cases, no 23 cases. Time to initial treatment time to first recurrence (PFS1) more than 12 months in 27 cases, less than 12 months in 8 cases. Combined treatment group (45 cases), aged 35-74 years old. Pathological types: serous adenocarcinoma in 30 cases, mucinous adenocarcinoma in 7 cases, endometrioid carcinoma in 4 cases, clear cell carcinoma in 4 cases. Pathological grading: G1 in 4 cases, G2 in 6 cases, G3 in 35 cases. Lymph node invasion: 16 cases, no 29 cases. PFS1 more than 12 months in 34 cases, PFS1 less than 12 months in 11 cases. There was no significant difference between the two groups in age, pathological type, pathological grade, lymph node invasion, PFS1 and other basic data (P>0.05). They can be compared.

2.2 Inclusion and exclusion criteria

Inclusion criteria: (1) The initial treatment regimen was cytoreductive surgery. (2) Are in line with the Chinese Medical Association in 2003 the standard diagnosis and treatment of recurrent ovarian cancer to develop Gynecological Oncology Group (recommended) diagnostic criteria for recurrence of ovarian cancer: The level of CA125 increased more than 100 U/mL, and increased exponentially. There were pleural effusion, ascites, physical examination and imaging examination[3]. (3) Cellular immunotherapy for the first time. (4) The expected survival time is more than 2 months. Informed consent was obtained from all patients, and informed consent was signed. Exclusion criteria: (1) Combined with other malignant tumors. (2) Other kinds of immunotherapy have been accepted in the past. (3) Routine blood tests and liver and renal function tests are not in accordance with the treatment requirements. (4) Patients with other serious organ diseases or acute or chronic infection.

2.3 Method

The patients in the chemotherapy group received conventional TC chemotherapy [paclitaxel (Taxinol) + carboplatin (Carboplatin)]: Paclitaxel 175 mg/m^2 + carboplatin 75 mg/m^2, intravenous infusion of 1D every cycle, 21 d for a cycle. Combined treatment group were given DC-CIK cell immunotherapy combined with TC chemotherapy; The two groups were treated with 21 d for a cycle, 3 cycles were treated.

DC-CIK cell immunotherapy: 7-10 d was collected from 50mL before chemotherapy, peripheral blood mononuclear cells were collected by centrifugation and separation, suspension was cultured in medium with (1.0-1.8)×10^7/L concentration. Recombinant human interferon γ (rh IFN-γ) was added. The final concentration was 3 000×10^6 U/L, and cultured in CO2 incubator at 37 and 5% volume concentration. After 24 h, the culture medium containing anti CD3 Mc Ab 50 μ g/L and Rh IL-2 1.5×10^5 U/L was added. At 37 ℃ and 5% CO2 concentration in the culture box to culture, every 3 d of the amount of fluid infusion. DC-CIK cells were collected by 7 d culture, and then returned to the hospital 1 times a day after qualified monitoring. The number of 2.4-4.0×10^7 cells was suspended in 100 mL Sodium Chloride Solution, and the infusion time was 0.5-1 h. The number of transfused cells was controlled according to the amount of amplification, and 6d was transfused back. Cell culture provided by Shanghai Ke Lessing Biotechnology Co. Ltd.CLS cell culture technology.

2.4 Observation index

Immunity: Before and after the treatment of 7 d by flow cytometry (America Beckman Coulter company’s FC500 flow cytometry) using fluorescent antibody assay in patients with peripheral blood T lymphocyte subsets and natural killer cells (NK), T cell subsets including T lymphocytes (CD3+) and T helper cells (CD3+CD4+), CD4+CD8+, regulatory T cells (CD4+CD25+).

Coagulation function: The blood coagulation function was detected by automatic turbidimetric analyzer (Sysmex CA7000) before and after the treatment of 7 d. Including prothrombin time (PT), thrombin time (TT), activated partial thromboplastin time (APTT), fibrinogen (FIB), platelet (PLT).

Tumor stem cell markers: The serum levels of CD133 and DDX4 were detected by double sandwich enzyme-linked immunosorbent assay (ELISA) kit before and after the treatment of 7 d. The kit and kit were provided by Shanghai Kang biological technology Co., Ltd., and the operation was carried out strictly according to the instructions of the kit.

2.5 Statistical methods

SPSS 21 was used for statistical analysis. The immune function, blood coagulation function and tumor stem cell marker level were verified by normal distribution and homogeneity of variance, and expressed by Mean ± SD. Group design t test was used to compare the same time points between the two groups, with P<0.05 as the difference was statistically significant.

Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>CD3+ (%)</th>
<th>CD3+CD4+ (%)</th>
<th>CD4+CD8+ (%)</th>
<th>CD4+CD25+ (%)</th>
<th>NK (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple chemotherapy group (n=35)</td>
<td>Before treatment</td>
<td>53.63±5.83</td>
<td>31.69±7.78</td>
<td>0.98±0.15</td>
<td>12.45±2.32</td>
<td>14.14±4.37</td>
</tr>
<tr>
<td></td>
<td>After the end of treatment 7 d</td>
<td>54.97±9.75</td>
<td>28.18±10.29</td>
<td>0.98±0.17</td>
<td>11.84±3.66</td>
<td>15.06±6.02</td>
</tr>
<tr>
<td>Combined therapy group (n=45)</td>
<td>Before treatment</td>
<td>55.75±10.20</td>
<td>34.94±9.40</td>
<td>1.06±0.25</td>
<td>11.66±2.18</td>
<td>13.51±5.08</td>
</tr>
<tr>
<td></td>
<td>After the end of treatment 7 d</td>
<td>64.82±9.21</td>
<td>41.67±10.22</td>
<td>1.57±0.41</td>
<td>8.05±2.67</td>
<td>25.50±6.21</td>
</tr>
</tbody>
</table>

Note: * said compared with the group before treatment P<0.05; ** said P<0.05 compared with the chemotherapy group after treatment.
3. Result

3.1. Comparison of immune function before and after treatment in patients

Before treatment, there was no significant difference in T lymphocyte subsets and NK cells in peripheral blood between the two groups (P>0.05). After the end of treatment 7 d, the CD3+ of the combined treatment group increased from (55.75 ± 10.20)% to (64.82 ± 9.21)%; CD4+ increased from (34.94 ± 9.40)% to (41.67 ± 10.22)%, CD4+/CD8+ increased from (1.06 ± 0.25)% to (1.57 ± 0.41)%. NK cells increased from (13.51 ± 5.08)% to (25.50 ± 6.21)%; CD3+CD4+CD25+ decreased from (11.66 ± 2.18)% to (8.05 ± 2.67)%, which was significantly lower than that before treatment, the differences were statistically significant (P<0.05). There was no significant difference in peripheral blood T lymphocyte subsets before and after treatment in the chemotherapy group (P>0.05). The improvement of peripheral blood T lymphocyte subsets and NK cells in the combined treatment group was better than that in the chemotherapy alone group (P<0.05). See Table 1 for details.

3.2. Comparison of coagulation function before and after treatment in patients

Before treatment, the two groups had no statistically significant peripheral blood coagulation index difference (P>0.05). 7 d after the end of treatment, the combined treatment group by FIB decreased from (3.81 ± 0.73) g/L to (3.47 ± 0.88) g/L, and the simple chemotherapy group by FIB decreased from (3.89 ± 0.72) g/L to (3.52 ± 0.81) g/L, compared with before treatment, the difference was statistically significant (P<0.05). The CD133 and DDX4 levels decreased from (139.47 ± 32.16) U/mL to (11.42 ± 3.01) U/mL, the CD133 decreased of the combined treatment group from (148.72 ± 34.28) U/mL to (11.42 ± 3.01) U/mL, and DDX4 decreased from (149.88 ± 32.17) U/mL to (13.49 ± 3.56) U/mL. They were significantly lower than before treatment, the difference was statistically significant (P<0.05). The CD133 and DDX4 levels of the combined treatment group were significantly lower than those of the chemotherapy group at the end of 7d, and the differences were statistically significant (P<0.05). See Table 2 for details.

3.3. Before and after treatment in patients with cancer stem cell markers in comparison

Before treatment, the two groups had no statistically significant difference between patients with CD133 and DDX4 (P>0.05). After the end of treatment 7 d, the CD133 of chemotherapy group decreased from (139.47 ± 32.16) U/mL to (25.35 ± 5.76) U/mL, and DDX4 decreased from (141.09 ± 30.29) U/mL to (33.96 ± 7.65) U/mL .the CD133 decreased of the combined treatment group from (148.72 ± 34.28) U/mL to (11.42 ± 3.01) U/mL, and DDX4 decreased from (149.88 ± 32.17) U/mL to (13.49 ± 3.56) U/mL. They were significantly lower than before treatment, the difference was statistically significant (P<0.05). The CD133 and DDX4 levels of the combined treatment group were significantly lower than those of the chemotherapy group at the end of 7d, and the differences were statistically significant (P<0.05). See Table 3 for details.

4. Discussion

Recurrent ovarian cancer as a high incidence and high mortality of malignant tumors has become an important problem to be solved in the current clinical. The recurrence of ovarian cancer is mainly related to clinical stage, pathological type, histological grade, residual lesion size, tumor associated antigen CA125 level, lymph node metastasis and immune function of patients[4,5]. At present, the treatment of recurrent ovarian cancer is mainly surgery, radiotherapy and chemotherapy. But the therapeutic effect is limited, and the adverse reactions such as immunosuppression caused by radiotherapy and chemotherapy are the important reasons leading to the poor prognosis of patients[6]. With the development and application of tumor biotherapy, immunotherapy, especially DC-CIK cell immunotherapy, plays an important role in tumor therapy[7].

DC-CIK cells are the effector cells of DC and CIK co-cultured with two kinds of cells, which have strong anti-tumor activity and regulate the immune function of the body. The occurrence of tumor is closely related to organism immunity. Under pathological condition, the immune function of tumor patients is suppressed, and the immunogenicity of tumor cells in vivo is reduced, which makes tumor cells escape from the immune system "surveillance", leading to the occurrence and development of tumors[8,9]. Cellular immunotherapy is to stimulate and amplify the immune cells in vitro, and then re-transport it back to the patient, so that it can play the role of killing tumor cells in vivo, so as to achieve the purpose of anti-tumor[10]. The main anti-tumor reaction is cellular immunity, and the main effect is T lymphocyte. By detecting the changes of CD3+, CD4+, CD8+ and NK cells in peripheral blood, the cellular immune function of human body can be reflected[11]. The results showed that the levels of T lymphocyte subsets in the peripheral blood of the combined treatment group were significantly improved compared with those before treatment, and the improvement effect was better than that of the chemotherapy alone group. This indicates that DC-CIK cell immunotherapy can effectively improve the immune function of patients with recurrent ovarian cancer, and improve the anti-tumor immune effect of patients. This is because...
chemotherapy can enhance the immunogenicity of tumor cells and increase the killing effect of T lymphocytes on tumor cells. However, due to the cytotoxicity and low selectivity of chemotherapeutic drugs, there is some damage or inhibition to the immune function of normal tissue and organism. The body lacks effector cells, and DC-CIK cell immunotherapy can give the immune support in time. Studies have shown that DC cells are resistant to paclitaxel. The suitable concentration of paclitaxel can promote the maturation of DC cells, and enhance the tumor antigen presenting effect so as to enhance the chemotherapy of tumor killing ability, reduce the damage of chemotherapy, improve immune function. In addition, long-term chemotherapy leads to drug resistance in tumor cells. Immunotherapy can reduce the drug resistance of tumor by attacking the over expression protein of tumor cell surface, thus enhancing the killing effect of chemotherapeutic drugs [12-14].

The process of proliferation and metastasis of tumor cells involves the activation of coagulation mechanism, and makes the patients often in hypercoagulable state. Studies have shown that more than 90% of patients with abnormal coagulation function, often manifested as PT, TT, APTT, FIB and PLT and other coagulation function related indicators of significant changes [15]. The shortening of PT, TT and APTT indicates different degrees of hypercoagulability and thrombosis. The increase of PLT suggests abnormal coagulation, thrombosis and disseminated intravascular coagulation. The level of FIB may predict tumor invasion, metastasis and prognosis [16]. But the results of this study show that the two groups after treatment only FIB level decreased significantly than that before treatment, and the difference of FIB level between the two groups after treatment had no significant difference. PT, TT, APTT was increased slightly compared with before treatment but no significant change. Which indicating that DC-CIK cell immunotherapy combined with chemotherapy did not significantly improve the coagulation function of recurrent ovarian cancer patients. In addition, chemotherapy itself can cause severe vascular endothelial damage, activate coagulation and cause coagulation disorders [17]. However, this study only observed the changes of coagulation function before treatment and after the treatment of 7D, and further subdivided the time and prolonged the study time to further observe the changes of coagulation function in patients with recurrent ovarian cancer.

CD133 and DDX4 are two important tumor stem cell markers [18]. Studies have shown that CD133 and DDX4 are highly expressed in ovarian cancer tissues, and their expression levels are related to the pathological characteristics of the tumor. Both of them are involved in the occurrence and development of ovarian cancer, and are of great significance for the diagnosis and prognosis evaluation of ovarian cancer [19-21]. In the study, the levels of CD133 and DDX4 in the two groups were significantly lower than those before treatment, and the combined treatment group was lower than that of the chemotherapy group. It suggests that the combination therapy group has a better prognosis, indicating that DC-CIK cell immunotherapy combined with chemotherapy can regulate the level of serum tumor stem cell markers in patients with recurrent ovarian cancer, thereby improving the prognosis of patients.

In summary, DC-CIK cell immunotherapy combined with chemotherapy in the treatment of recurrent ovarian cancer can significantly enhance the immunity of patients, improve the level of FIB, and regulate the level of serum tumor stem cell markers. Which has positive significance in improving the prognosis of patients. It is worthy of clinical application.

Reference