Effect of DC-CIK in combined with chemotherapy on the immunological function in patients with advanced non-small cell lung cancer

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Objective: To explore the effect of DC-CIK in combined with chemotherapy on the immunological function in patients with advanced non-small cell lung cancer (NSCLC).

Methods: A total of 86 patients with NSCLC who were admitted in our hospital were included in the study and randomized into the observation group and the control group with 43 cases in each group. The patients in the two groups were given routine chemotherapy. On this basis, the patients in the observation group were given DC-CIK cellular immunotherapy. The patients in the two groups were treated for 3 courses. The peripheral blood T lymphocytes and cytokines after treatment in the two groups were compared. The clinical efficacy and adverse reactions in the two groups were evaluated.

Results: The total effective rate and disease control rate after treatment in the observation group were slightly higher than those in the control group, but the comparison between the two groups was not statistically significant. CD8+ after treatment in the observation group was significantly reduced, while CD3+, CD4+, and CD4+/CD8+ were significantly elevated when compared with before treatment. CD3+ and NK contents, and CD4+/CD8+ after treatment in the observation group were significantly higher than those in the control group. IFN-γ, IL-4, TNF-α levels, and Th1/Th2 after treatment in the observation group were significantly higher than those in the control group, while TGF-β level was significantly lower than that in the control group. The occurrence rate of nausea and vomiting, and liver and renal function damage in the observation group was significantly lower than that in the control group, while the median progression free survival (PFS) was significantly longer than that in the control group, and 2-year survival rate was significantly higher than that in the control group.

Conclusions: DC-CIK in combined with chemotherapy can correct the immunologic disorder in patients with advanced NSCLC, and strengthen the anti-tumor effect.

1. Introduction

Non-small cell lung cancer (NSCLC) is located in the first place among the death of cancer, accounting for about 80%-85% of lung cancer[1]. Due to radioactive cell lines resistance and drug resistance in partial patients, the therapeutic effect of advanced chemoradiotherapy is poor. The adoptive immunotherapy is a new type anti-tumor treatment mode after surgery, chemotherapy, and radiotherapy, and has achieved a preferable effect in the treatment of tumors[2-5]. Dendritic cell (DC) is a kind of antigen presenting cell, and can activate the specific anti-tumor response. Cytokine induced killer cells (CIK) are the heterogeneous immunologic effector cells, mainly including CD3+ and CD56+ T cells, and can inhibit the growth of various tumors. After co-cultivation, DC and CIK have a strong and synergistic anti-tumor effect[6]. The study is aimed to explore the effect of DC-CIK in combined with chemotherapy on the immunological function in patients with advanced NSCLC.
2. Materials and methods

2.1. Clinical materials

A total of 86 patients with NSCLC at stage I-IV were included in the study. Inclusion criteria: (1) those who were confirmed with NSCLC by chest CT, MRI, and histopathology before operation; (2) those who had not taken chemotherapy, radiotherapy, or other related treatments; (3) those whose KPS≥60, predicted survival time >3 months, and could receive long-term follow up; (4) those who had no history of diabetes and heart disease; (5) those whose blood routine examination, ECG, and liver and kidney function were basically normal before operation. Exclusion criteria: (1) those who were merged with intracranial metastasis or other malignant tumors; (2) those who were merged with severe cardiovascular, liver and kidney, endocrine system, and hematological system diseases; (3) those who had acute and chronic infections; (4) those who had the history of mental disorders; (5) those who were pregnant or in the lactation period. The patients were randomized into the observation group and the control group with 43 cases in each group. In the observation group, 30 were male, and 13 were female; aged from 35 to 68 years old; 17 had adenocarcinoma, 24 had squamous carcinoma, and 2 had adenosquamous carcinoma and others; 10 at stage II, 16 at stage IIIa, 12 at stage IIIb, and 5 at stage IV according to TNM staging. In the control group, 28 were male, and 15 were female; aged from 32 to 70 years old; 18 had adenocarcinoma, 22 had squamous carcinoma, and 3 had adenosquamous carcinoma and others; 9 at stage II, 18 at stage IIIa, 13 at stage IIIb, and 3 at stage IV according to TNM staging. The comparison of gender, age, disease staging, and other general materials between the two groups was not statistically significant (P>0.05), but it was comparable.

2.2. Methods

2.2.1. DC-CIK cell culture

A volume of 100 mL peripheral blood mononuclear cell was collected in sterile 2 h before chemotherapy in each cycle in the observation group, and incubated at 37 ℃ for 2 h. The suspension cell was extracted for CIK cell culture, while the adherent cell was used for DC cell culture. In vitro induction of GM-CSF (1 000 U/mL), IL-4 (1 000 U/mL), and TNF-α (1 000 U/mL) was used for DC. In vitro induction of IL-2 (1 000 U/mL), CD-3 (0.5 μg/mL), and IFN-γ (1 000 U/mL) was used for CIK. The cells were performed with phenotype identification, cell counting, and sterility detection before retransfusion.

2.2.2. Treatment methods

The patients in the two groups were given gemcitabine (1 250 mg/m²), ivdrip, on d1, and meanwhile given hydration, diuresis, liver protection, and other routine treatments. Blood routine examination, and liver and renal functions were regularly rechecked. 21 d-treatment was regarded as one course, continuously for 3 courses. In the observation group, DC-CIK cell was cultured for 14 d, and centrifuged. The cells were collected and washed. After resuspension with 100 mL normal saline, cells were retransfused for 2 h, qd, for 5 times.

2.3. Observation indicators

The short-term and long-term clinical efficacy after treatment in the two groups was compared. The short-term efficacy was divided into CR, PR, SD, and PD according to WHO RECIST. ORR=(CR+PR) number/total number ×100%, DCR=(CR+PR+SD) number/total number ×100%. Long-term efficacy: OS was from the confirmation day to death day or follow-up deadline day; PFS was from the confirmation day to recurrence, metastasis or follow-up deadline day. Follow-up time: minimum for 7 months, maximum for 25 months, last follow-up time was until February, 2016. The immunological function before and after treatment in the two groups was compared. FCM was used to detect the peripheral blood T lymphocyte subsets. ELISA was used to detect the expression levels of cytokines. Th1/Th2 was calculated. The adverse reactions in the two groups were compared. The adverse reactions during the treatment process were evaluated according to NCI-CIT.

2.4. Statistical analysis

SPSS 22.0 software was used for the statistical analysis. The measurement data were expressed as mean ± SD. The paired t test was used for the intra-group comparison, and the independent t test was used for the comparison between the two groups. The enumeration data were expressed as percentage, and chi-square test was used. Kaplan-Meier survival analysis was used to calculated the survival time, and log-rank test was used. P<0.05 was regarded as statistically significant.

3. Results

3.1. Comparison of the short-term efficacy

No CR was in the two groups. ORR and DCR in the observation group were 37.21% and 86.05%, respectively, while those in the control group were 30.23% and 72.09%, respectively. The comparison of ORR and DCR between the two groups was not statistically significant (P>0.05) (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>CR</th>
<th>PR</th>
<th>SD</th>
<th>PD</th>
<th>ORR(%)</th>
<th>DCR(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>43</td>
<td>0</td>
<td>16</td>
<td>21</td>
<td>6</td>
<td>37.21</td>
<td>86.05</td>
</tr>
<tr>
<td>Control</td>
<td>43</td>
<td>0</td>
<td>13</td>
<td>18</td>
<td>12</td>
<td>30.23</td>
<td>72.09</td>
</tr>
</tbody>
</table>
Table 2.
Comparison of the peripheral blood T lymphocyte subsets before and after treatment between the two groups (%).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>CD3⁺</th>
<th>CD4⁺</th>
<th>CD8⁺</th>
<th>CD4⁺/CD8⁺</th>
<th>NK</th>
<th>Treg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>43</td>
<td>Before treatment</td>
<td>53.32±8.66</td>
<td>30.29±8.48</td>
<td>25.69±6.58</td>
<td>1.17±0.19</td>
<td>12.73±5.18</td>
<td>6.61±3.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>59.46±7.64*</td>
<td>36.12±9.18*</td>
<td>22.67±6.13*</td>
<td>1.59±0.23*</td>
<td>14.83±7.22*</td>
<td>4.11±2.62*</td>
</tr>
<tr>
<td>Control</td>
<td>43</td>
<td>Before treatment</td>
<td>53.69±9.57</td>
<td>31.22±7.16</td>
<td>26.29±6.59</td>
<td>1.16±0.21</td>
<td>11.43±5.19</td>
<td>6.69±3.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>55.53±7.69</td>
<td>35.27±8.14*</td>
<td>24.96±6.15</td>
<td>1.41±0.18*</td>
<td>9.35±6.23</td>
<td>5.67±2.31</td>
</tr>
</tbody>
</table>

*p<0.05, when compared with before treatment; *p<0.05, when compared with the control group.

Table 3.
Comparison of the peripheral blood cytokines before and after treatment between the two groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>IFN-γ</th>
<th>IL-4</th>
<th>TNF-α</th>
<th>TGF-β</th>
<th>Th1/Th2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>43</td>
<td>Before treatment</td>
<td>37.28±3.12</td>
<td>28.17±2.83</td>
<td>6.34±2.36</td>
<td>1.67±0.46</td>
<td>1.30±0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>45.72±3.82*</td>
<td>31.25±2.87*</td>
<td>7.56±2.36*</td>
<td>1.36±0.32*</td>
<td>1.48±0.33*</td>
</tr>
<tr>
<td>Control</td>
<td>43</td>
<td>Before treatment</td>
<td>37.14±3.25</td>
<td>28.22±2.69</td>
<td>6.32±3.88</td>
<td>1.67±0.44</td>
<td>1.31±0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After treatment</td>
<td>29.38±3.66*</td>
<td>21.63±2.29*</td>
<td>3.52±1.12*</td>
<td>1.49±0.27*</td>
<td>1.34±0.29</td>
</tr>
</tbody>
</table>

*p<0.05, when compared with before treatment; *p<0.05, when compared with the control group.

3.2. Comparison of the peripheral blood T lymphocyte subsets before and after treatment between the two groups

The comparison of the peripheral blood T lymphocyte subsets before treatment between the two groups was not statistically significant (P>0.05). CD8⁺ after treatment in the observation group was significantly reduced, while CD3⁺, CD4⁺, and CD4⁺/CD8⁺ were significantly elevated when compared with before treatment (P<0.05). CD4⁺ and CD4⁺/CD8⁺ after treatment in the control group were significantly elevated when compared with before treatment (P<0.05). CD3⁺ and NK contents, and CD4⁺/CD8⁺ after treatment in the observation group were significantly higher than those in the control group (P<0.05) (Table 2).

3.3. Comparison of the peripheral blood cytokines before and after treatment between the two groups

The comparison of the peripheral blood cytokines before treatment between the two groups was not statistically significant (P>0.05). IFN-γ, IL-4, TNF-α levels and Th1/Th2 after treatment in the observation group were significantly elevated when compared with before treatment (P<0.05), while TGF-β level was significantly lowered when compared with before treatment (P<0.05). The occurrence rate of nausea and vomiting, and liver and renal function damage in the observation group was significantly lower than that in the control group (P<0.05) (Table 4).

3.4. Comparison of the adverse reactions

The occurrence rate of nausea and vomiting, and liver and renal function damage in the observation group was significantly lower than that in the control group (P<0.05) (Table 4).

3.5. Comparison of the long-term efficacy

The median PFS in the observation group was 8.36 months, and median OS was 14.58 months, while those in the control group were 5.27 months and 12.11 months, respectively. The comparison of median PFS between the two groups was statistically significant (P<0.05), but the comparison of median OS between the two groups was not statistically significant (P>0.05). The 2-year survival rate in the observation group (32.56%, 14/43) was significantly higher than that in the control group (13.955%, 6/43) (P<0.05).

4. Discussion

Currently, the chemoradiotherapy with platinum-based drugs is the first line treatment protocol for the treatment of progressive lung cancer. With the development of biotechnology, the molecular target drug is widely applied in the treatment of advanced lung cancer, but it still has primary drug resistance and poor prognosis, with low 5-year survival rate. The adoptive immunotherapy is a new type treatment mode on the basis of strengthening the ability of killing tumor cells. Currently, DC-CIK in combined with platinum-based are widely applied in the clinic.

Some researches demonstrate that OS and DCR in patients with...
advanced NSCLC treated by GP are superior to those by paclitaxel, vinorelbine, and docetaxel, and GP is also the first line treatment protocol for advanced NSCLC recommended by NCCN Guideline (2012)[7]; therefore, GP is adopted in the study for the basic treatment. The results in the study showed that ORR and DCR in the observation group were slightly higher than those in the control group, but the comparison was not statistically significant (P>0.05), which is consistent with the results reported by Ye et al and Wei et al[8,9]. While in some researches[10,11], DC-CIK immunotherapy was performed, and the results showed that the effective rate in the treatment group was significantly higher than that in the control group (P<0.05), which can be explained by that the differences of the patients themselves (gender, age, TNM staging, and disease types), and the adopted chemotherapy and immunotherapy can pose a great effect on the disease progression, which can cause different therapeutic effects.

After chemotherapy, partial immune cells are killed with the tumor cells; therefore, the immunological function is inhibited to a certain degree. DC can strengthen T cell response, activate NK cell, and magnify the anti-tumor effect. CIK can specifically kill NK cells, has non-MHC restrictive killing ability, and precisely and effectively kill the tumor cells in a condition of not damaging the normal cells. DC-CIK combined therapy can reduce the recurrence and metastasis of tumors, with a preferable safety. Some researches demonstrate that the peripheral blood CD8+T cells are increased in patients with advanced NSCLC, and the proportion of CD4+ and CD8+T cells is out of balance, which is probably associated with NSCLC progression[12]. Treg is a kind of T cell with immunosuppression. The peripheral blood Treg cells are increased in patients with advanced NSCLC. Some researches demonstrate that the condition in tumor patients with increased Treg cells is more serious, and the disease progression time is shorter[13]. The results in the study showed that CD8+ after treatment in the observation group was significantly reduced, while CD3+, CD4+, and CD4+/CD8+ were significantly elevated when compared with before treatment (P<0.05), and the variation range was significantly higher than that in the control group (P<0.05); meanwhile, CD3+ and NK contents, and CD4+/CD8+ after treatment in the observation group were significantly higher than those in the control group (P<0.05); Treg content after treatment in the two groups was reduced when compared with the before treatment, but the comparison was not statistically significant (P>0.05), indicating that the combined treatment can destroy the inactivation and resistance of tumor cells through inhibiting the immunosuppression of CD8+T cells in order to play the anti-tumor effect. The results in the study showed that IFN-γ, IL-4, TNF-α levels, and Th1/Th2 after treatment in the observation group were significantly elevated, while TGF-β level was significantly reduced when compared with before treatment (P<0.05), and the variation degree was significantly superior to that in the control group (P<0.05), indicating that the combined treatment can inhibit the expressions of TGF-β, and other immunosuppression factors, reverse Th2 to Th1, and regulate the balance of Th1 and Th2 in order to enhance the immune cell activity, and strengthen the immune killing function, with a more significant treatment advantage.

In conclusion, DC-CIK in combined with chemotherapy have a preferable application prospect, and can be served as a new mode for the treatment of advanced NSCLC.

References