Effect of compound Kushen injection on T-cell subgroups and natural killer cells in patients with locally advanced non-small-cell lung cancer treated with concomitant radiochemotherapy

Zhao Zhongquan, Liao Hehe, Ju Ying

Abstract
OBJECTIVE: To observe effect of compound Kushen injection on T-cell subgroups and NK cells in patients with locally advanced non-small-cell lung cancer (NSCLC) treated with concomitant radiochemotherapy.

METHODS: We randomly divided 60 patients with locally advanced NSCLC who were treated at our hospital between May 2011 and May 2013 into a treatment group and a control group by drawing. The treatment group (n = 30) received concomitant radiochemotherapy plus compound Keshen injection, and the control group (n = 30) received only radiochemotherapy.

RESULTS: After treatment, levels of CD3+, CD4+, CD4+/CD8+ and CD16+/CD56+ cells had significantly increased, and CD8+ cells had significantly decreased, in the treatment group compared with both their pretreatment levels and with levels in the control group. In the control group, post-treatment levels of CD3+, CD4+, CD4+/CD8+ and CD16+/CD56+ cells were not significantly changed from pretreatment levels. The two groups did not significantly differ in their rates of toxicity reactions (P > 0.05).

CONCLUSION: Compound Kushen injections can increase immunologic function in patients with locally advanced non-small cell lung cancer who receive concomitant radiochemotherapy.

INTRODUCTION
Patients with non-small cell lung cancer (NSCLC) account for about 80% of patients with lung cancer. Among them, locally advanced NSCLC (LA-NSCLC) accounts for 40%-50% of NSCLC. For LA-NSCLC, successful excision is less probable, and concomitant radiochemotherapy (CRCT) is the primary treatment at present. However, compared with single-mode radiotherapy or chemotherapy, CRCT increases toxicity and decreases immunologic function, leading to decreased tolerance that may curtail treatment in severe cases. Traditional Chinese Medicine holds that in treating tumors, eliminating pathogenic factors and strengthening genuine Qi are of equal importance; eliminating pathogenic factors is killing tumor cells by using radiotherapy or chemotherapy, and strengthening genuine Qi protects immunologic functions of the organism by...
using drugs, increasing immunity of the organism.\textsuperscript{24} Clinically, Chinese drugs with functions of strengthening genuine Qi to consolidate the constitution, promoting blood circulation to remove blood stasis, and clearing heat and removing toxic substance are more used for strengthening genuine Qi at present. Compound Kushen injection (CKI) is made from the extract of Kushen (\textit{Radix Sophorae Flavescentis}) and Tufuling (\textit{Rhizoma Smilacis Chinae}), containing the chemical components such as oxymatrine, matrine, sophocarpine, sopherine, sopheridine, and kurarine.\textsuperscript{7} Modern study indicates that CKI has many pharmacologic properties, such as anti-tumor, anti-inflammation, analgesia, and immunity-enhancing activity, and is widely used for accessory treatment of cancers including that of the digestive tract, NSCLC, and primary liver cancer. Since January 2011, our department has used CKI to increase immunity of patients with LA-NSCLC with CRCT, achieving a good clinical therapeutic effect, as reported in the following.

**MATERIALS AND METHODS**

**Clinical data**

We selected 60 patients (32 men and 28 women) with LA-NSCLC who were treated at our hospital between May 2011 and May 2013. They were assigned to a treatment group or a control group ($n = 30$ for both), by using a random drawing. This experimental study was approved by the Ethics Committee of the Hospital. All of the patients provided signed informed consent forms.

**Inclusion criteria**

We included patients for whom (a) NSCLC was established as adenocarcinoma or squamous carcinoma, by pathological or cytological detection after aspiration biopsy; (b) the patient had phase III a or III b disease (IASLC2009 staging criteria); \textsuperscript{5} (c) The patients had not received previous radiotherapy or chemotherapy; (d) patient’s Karnofsky performance score (KPS) $\geq 70$, and predicted living time $\geq$ a half year; (e) electrocardiogram, routine blood tests and liver function and renal function were normal; and (f) the patient provided informed consent.

**Exclusion criteria**

We excluded patients who had (a) obvious pulmonary emphysema or respiratory function decompensation, (b) autoimmune disease, or (c) phase IIIa disease that required surgery.

**Treatment methods**

Both the control and treatment groups received 3-dimensional conformal radiation therapy (3D-CRT) with a concomitant regimen of duoxitasai + cisplatin (DP). The treatment group received CKIs at the same time.

For the 3D-CRT: The body position was fixed with a body model, with Toziba 16-row CT modeling location and scanning layer thickness 5 mm, and the figure was transferred into the planning system (3DTPS). Clinical target area volume (CTV) included the primary focus of the lung, the hilus of lung of the same side and the draining area of mediastinal lymph nodes. The margin was 0.6-0.8 cm outside the tumor volume (GTV), shown by the lung window in the CT slice. The planned target volume (PTV) was extra-spreading 0.8-1.5 cm on the basis of CTV; 95% of the isodose curve covered the PTV, with lung dose: V20 $\leq$ 20%; heart: $\leq$ 30 Gy; spinal cord: $\leq$ 40 Gy. Patients received 6MV-X radiation therapy at 4-6 conformal visual fields, 2.0 Gy/f, 1 f/d, 5 f/w; after the tissue dosage reached to 40 Gy/4 w, chest CTs were rechecked. According to any changes of the focus, the primary focus was then radiated with DT 60-70 Gy over the reduced area.

The DP (duoxitasai + cisplatin) regimen was Duoxitasai (Jiangsu Hengrui Medical Limited Company, Batch No. H20031227, Lianyungang, China) 20 mg/m², intravenous drip, on the first day; cisplatin (Jiangsu Haoshen Pharmacy Limited Company, Batch No. H20050563, Lianyungang, China) 30 mg/m², intravenous drip on the first day. The chemotherapy was concomitantly carried out on the first day of radiotherapy, once a week, until the end of radiotherapy. During chemotherapy, patients received treatments for inhibiting acid and nausea, and protecting the liver.

In the treatment group, at the beginning of radiochemotherapy, compound Kushen injection (Shanxi Zhen-dong Pharmacy Limited Company, Chinese medicine permit No. Z14021231) 20 mL plus 200 mL saline was given, intravenous drip, at 40-60 drips each min, once a day for 15 days.

**Observation indices**

Patients’ peripheral blood T-lymphocyte subgroups (CD3+, CD4+, CD8+) and natural killer (NK) cells (CD16+, CD56+) were detected with a flow cytometry one day before treatment and one week after the end of the treatment in both groups. Ratios of CD4+/CD8+ were calculated.

**Statistical analysis**

Statistical analysis was carried out with SPSS13.0 software (SPSS Inc., Chicago, IL, USA). Data were expressed as mean $\pm$ standard deviation ($\bar{x} \pm s$). Independent sample $t$-tests were used to compare the two groups. Toxicity reactions in the two groups were compared with $\chi^2$ tests. $P < 0.05$ was considered significance.

**RESULTS**

**Comparison of basic data of the patients**

At last, 60 patients (32 men and 28 women) with
LA-NSCLC were included in our study. Thirty patients were assigned in treatment group and control group, respectively. The two groups did not significantly differ in clinicopathological informations \((P > 0.05, \text{ Table 1})\).

**Changes of T-cell subgroups and NK cells**

One day before treatment, the treatment and control groups did not significantly differ in the peripheral blood counts for CD3+, CD4+, CD8+, CD4+/CD8+ cells or NK cells (CD16+/CD56+) \((P > 0.05)\). After the end of treatment, levels of CD3+, CD4+, CD4+/CD8+ and CD16+/CD56+ cells increased and CD8+ cells decreased significantly in the treatment group (Figures 1-3), compared with both their pretreatment levels and levels in the control group \((both P < 0.05)\).

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment group (n = 30)</th>
<th>Control group (n = 30)</th>
<th>(\chi^2) value</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>18</td>
<td>14</td>
<td>1.071</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>12</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Age &lt; 60 years</td>
<td>17</td>
<td>11</td>
<td>2.411</td>
<td>0.121</td>
</tr>
<tr>
<td>Age ≥ 60 years</td>
<td>13</td>
<td>19</td>
<td>0.625</td>
<td>0.732</td>
</tr>
<tr>
<td>KPS 70-90</td>
<td>16</td>
<td>19</td>
<td></td>
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<td>KPS ≥ 90</td>
<td>14</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pathological type</td>
<td>Squamous carcinoma</td>
<td>15</td>
<td>17</td>
<td>0.268</td>
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<tr>
<td></td>
<td>Adenocarcinoma</td>
<td>15</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>TNM phase II a</td>
<td>16</td>
<td>12</td>
<td>1.071</td>
<td>0.301</td>
</tr>
<tr>
<td></td>
<td>II b</td>
<td>14</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

Notes: both the control and treatment groups received 3D-CRT with a concomitant regimen of DP. Differently, the treatment group received CKIs at the same time. KPS: Karnofsky performance score; TNM: TNM staging; 3D-CRT: 3-dimensional conformal radiation therapy; CKIs: compound Kushen injection; DP: duoxitasai + cisplatin.

![Figure 1](image1.png)  
*Figure 1 Change of CD3+ count in the peripheral blood of the treatment group  
A: before treatment; B: after treatment.*

![Figure 2](image2.png)  
*Figure 2 Changes of CD4+ and CD8+ counts in the peripheral blood of the treatment group  
A: before treatment; B: after treatment.*
implies that immunologic function in patients with LA-NSCLC with CRCT after CKI significantly improved. In the control group, pre- and post-treatment CD3+, CD4+, CD4+/CD8+ and CD16+/CD56+ levels did not significantly differ (P > 0.05; Table 2).

Toxicity reactions
Toxicity reactions were assigned scores of I-IV in both the treatment and control groups. Among them, decreased white cells were scored as grade I: 3.0 × 10^9/L; grade II: 2.0-2.9 × 10^9/L; grade III: 1.0-1.99 × 10^9/L; and grade IV: < 1.0 × 10^9/L. Hemoglobin decrease was scored as grade I: 95-109 g/L; grade II: 80-95 g/L; grade III: 65-79 g/L; and grade IV: < 65 g/L. Blood creatinine levels > 133 μmol/L were considered to indicate injured renal function. Transaminase activity above 40 U/L was considered to indicate injured liver function. The two groups did not significantly differ in toxicity incidence rates (Table 3). In the treatment group, one patient had fever after CKI (highest body temperature: 38.1°C); after symptomatic treatment, his temperature returned to normal, with no allergy and other adverse responses, and no treatment-related death.

DISCUSSION
As CRCT can improve the survival time for LA-NSCLC, it has become a standard treatment program for middle-late NSCLC. However, radiotherapy and chemotherapy can not avoid injury to the immune system, which mainly manifests in the following two aspects: radioactivity has a directly cytotoxic action on immune effector cells, and the drugs of chemotherapy can directly or indirectly kill immunologic effector cells, both leading to decreased immune function. Thus, protecting immune functions of patients with LA-NSCLC is a consideration in CRCT.

Table 2 Comparison of T-cell subgroups and NK cells in the treatment and control groups before and after treatment (%; ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>CD3+</th>
<th>CD4+</th>
<th>CD8+</th>
<th>CD4+/CD8+</th>
<th>CD16+/CD56+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>30</td>
<td>62.9±3.1</td>
<td>31.4±4.3</td>
<td>31.1±5.2</td>
<td>1.0±0.8</td>
<td>11.4±1.4</td>
</tr>
<tr>
<td>Before treatment</td>
<td>30</td>
<td>72.2±3.0</td>
<td>38.2±2.3</td>
<td>22.2±1.0</td>
<td>1.7±2.2</td>
<td>18.1±0.7</td>
</tr>
<tr>
<td>After treatment</td>
<td>30</td>
<td>62.9±2.6</td>
<td>32.9±4.1</td>
<td>32.0±5.0</td>
<td>1.0±0.8</td>
<td>12.1±1.1</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>62.1±1.3</td>
<td>32.3±2.1</td>
<td>32.0±3.5</td>
<td>1.0±0.6</td>
<td>12.0±2.6</td>
</tr>
</tbody>
</table>

Notes: both the control and treatment groups received 3-dimensional conformal radiation therapy with a concomitant regimen of duoxita-sai + cisplatin. Differently, the treatment group received compound Kushen injection at the same time. Two dependent sample t-tests were used, compared with the control group after treatment, ′P < 0.05. Paired t-test was used, compared with the treatment group before treatment, ′′P < 0.05; compared with the control group before treatment, ′′′P > 0.05.

Table 3 Comparison of toxicity incidence rates between the treatment and control groups (%)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Nausea, vomiting</th>
<th>Leukopenia</th>
<th>Anemia</th>
<th>Radiation pneumonia</th>
<th>Impairment of renal function</th>
<th>Impairment of liver function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>30</td>
<td>17 (56.7)</td>
<td>14 (46.7)</td>
<td>10 (33.3)</td>
<td>2 (6.7)</td>
<td>0 (0.0)</td>
<td>3 (10.0)</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>19 (63.3)</td>
<td>13 (43.3)</td>
<td>11 (36.7)</td>
<td>1 (3.3)</td>
<td>2 (6.7)</td>
<td>4 (13.3)</td>
</tr>
</tbody>
</table>

Notes: both the control and treatment groups received 3-dimensional conformal radiation therapy with a concomitant regimen of duoxitasai + cisplatin. Differently, the treatment group received compound Kushen injection at the same time.

Figure 3 Change of NK cell count in the peripheral blood of the treatment group
A: before treatment; B: after treatment.
The combination of CKI with cyclophosphamide can promote transformation of T lymphocytes, improve maladjustment of CD4+/CD8+ ratio, strengthen activity and improve the NK killing rate.11 Dong et al12 treated 40 patients with advanced NSCLC using CKI with a PD (docetaxel + cisplatin) chemotherapy regimen; they found the ratio of T cell subgroups and NK cells significantly improved, increasing patients’ immunologic function. Hong et al13 randomly divided 50 patients with middle-late lung adenocarcinoma into a CKI plus chemotherapy group (treatment group) and a chemotherapy-only group (control group). The percentage of CD4+ were 39.7% ± 1.7% in treatment group and 31.5% ± 1.4% in control group after treating (P < 0.001). And percentage of CD8+ were 27.8% ± 1.3% in treatment group and 24.3% ± 1.6% in control group (P < 0.001). Research results of Luo14 were similar to the results of Dong et al11 and Hong et al.13 However, at present, reports on the use of CKI in patients treated with LA-NSCLC with CRCT was not found. Therefore, this study was very necessary to carry out.

Anti-tumor immune responses are T cell-mediated specific anti-tumor immune response. T cells can be divided into CD4+ helper/induced T cells, CD8+ suppressor/killer T cells, at CD4+/CD8+ ratio of 1.5-2.0. When immunologic function in cancer patients is injured, CD4+ cells will decrease, CD8+ cells increase, and the CD4+/CD8+ decrease, or even invert.15 CD3+ can reflect the total levels of CD4 and CD8. CD16 and CD56 are surface markers of NK cells; when cellular immunologic function decreases, the ratio of NK cells will also decrease, unable to effectively kill tumor cells. Therefore, determination of changes of peripheral blood T cell subgroups and NK cells with flow cytometer can show changes of immunologic function.

In the present study, after the end of CRCT, T-cell subgroups and NK cells decreased somewhat in the control group patients compared with their pretreatment levels, indicating that radiochemotherapy has an inhibitory action on immunologic function; whereas among patients in the treatment group, T cell subgroups (CD3+, CD4+, CD4+/CD8+) and NK cells (CD16+ CD56+) significantly increased, which suggests that their immunologic function improved, which is similar to previous research results. Although CKI can increase immunologic function of the patient, whether it can prolong the survival of patients with LA-NSCLC treated with CRCT needs further investigation.

REFERENCES


