Effect of Xuebijing injection on peripheral T-lymphocyte subpopulations in patients with severe trauma

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【Abstract】Objective: To investigate the effect and clinical significance of Xuebijing injection on peripheral T-lymphocyte subpopulations in patients with severe trauma.

Methods: Thirty-three patients with severe trauma were randomly divided into a control group (n=16) and a treatment group (n=17). The patients of two groups were all treated conventionally, and the only difference was that Xuebijing injection was given to patients of the treatment group. The CD4+ and CD8+ subpopulations of T-lymphocyte in the peripheral blood were detected respectively on admission, 3rd and 5th days after trauma by double antibody labeling and flow cytometry.

Results: The CD4+ T-lymphocytes and CD4+/CD8+ ratio of peripheral blood in patients with severe trauma decreased markedly on the 3rd and 5th days after trauma. Furthermore, compared with control group, the peripheral CD4+ T-lymphocytes and CD4+/CD8+ ratio of treatment group renewed obviously on the 5th day after trauma, and showed statistical differences (P<0.05).

Conclusion: In the treatment of patients with severe trauma, the early use of Xuebijing injection is effective in correcting disorder or suppression of T-lymphocyte subpopulations regulating network, and promoting a more balanced profile of immunologic function.

Key words: Wounds and injuries; T-lymphocyte, helper-inducer; Flow cytometry; Medicine, Chinese traditional

With the development of the society, the incidence of trauma is progressively increased and trauma turns to be more severe and complex. According to statistics, trauma is the main cause for disability (people under the age of 65 years) and death (people under the age of 45 years).

Serious injury perturbs the immune system resulting in progressive dysfunction of immune responses during the first week after trauma. These disorganized immune responses are thought to contribute significantly to the development of sepsis and multiorgan dysfunction syndrome (MODS), which are the leading causes of death in patients who survive the initial injury.1-6 Regulated inflammatory responses are generally considered as a beneficial host response to injury, while posttraumatic hyperinflammation and ensuing immune incompetence are considered to be maladaptive and often auto-destructive.1,6

In the recent traumatic immunomodulatory study, the traditional Chinese medicine becomes highly emphasized for its therapeutic concepts of integrated and balanced regulation. Xuebijing is an intravenous injection consisting of 5 traditional Chinese medicines (Flos Carthami, Radix Paeoniae Rubra, Radix Salviae Miltiorrhiae, Radix Angelicae Sinensis, Ligusticum Chuanxiong Hort) selected out from 36 traditional Chinese herb compound formulas. A series of animal experiments have revealed that Xuebijing injection has some favorable immunomodulatory effects.7-11

Although the immune response to trauma is complex and involves all inflammatory cells, T-lymphocytes perform an important regulatory function and may play a major role in the dysfunctional regulation of immune function after trauma. Thus, the current clinical study was undertaken to investigate the effect of Xuebijing injection on regulating the immuno-inflammatory net-
work after trauma by dynamically determining the changes of peripheral T-lymphocyte subpopulations in patients with severe trauma.

**METHODS**

**Study population**

This study was approved by the Ethics Committee of Medical College, Zhejiang University, China. Thirty-three patients with severe trauma hospitalized between January 2006 and September 2007 were selected and the criteria were as follows: (1) According to the operation records and final diagnosis of patients, the extent of injuries was assessed using the Injury Severity Score (ISS), an anatomical scoring system that provides an overall score for the traumatic patients, and the patient’s ISS scores were greater than or equal to 20. (2) The patients were actively treated and did not die within 72 hours after trauma. (3) The patients were in good health condition previously and had no history of heart, lung, brain, liver, kidney, metabolic or immune diseases. (4) All subjects included in the study gave their informed consent.

Of the series, there were 23 male patients and 10 female patients, aged 19-59 years (mean=38.6 years). Nineteen patients were injured by traffic accidents, 8 by falling from a height, 3 by sharp instruments, 2 by blunt ones, and 1 by crush.

**Grouping and therapeutic scheme**

Patients in this series were randomly divided into a control group and a treatment group. In the control group, 16 patients (12 males and 4 females, aged 20-56 years) were treated after admission according to the traumatic conditions, such as by performing emergency operations, preventing infection, providing nutritional and multiple organ support, maintaining electrolytes and acid-base equilibrium, and controlling hyperglycemia. In the treatment group, 17 patients (11 males and 6 females, aged 19-59 years) received intravenous infusion of 50 ml Xuebijing (Tianjin Chase Sun Pharmaceutical CO. LTD, China; Batch number: 060818) plus 250 ml normal saline on the day of injury, twice a day for 5 days. Other treatment regimens were the same as the control group.

As depicted in Table 1, the gender distribution, age and ISS score of the patients in two groups were compared by Student’s t test and Fisher exact probability test, and the differences showed no statistical significance (P>0.05).

**Collection of blood samples**

By using EDTAK2 anticoagulation, peripheral venous blood samples (2 ml per sample) were taken from all the patients on admission, the 3rd and 5th days, respectively for determining the CD_{4}^{+} and CD_{8}^{+} subpopulations of T-lymphocytes.

**Flow cytometry analysis**

T-lymphocyte surface receptors were determined by double antibody labelling and flow cytometry. T-lymphocyte subpopulations analyzed included CD_{4}/helper and CD_{8}/suppressor.

Fresh anti-coagulating blood (100 µl) was taken and incubated at room temperature in the dark with an equal volume of fluorescence-labelled monoclonal antibodies (FITC-IgGl/PE-IgGl, FITC-CD_{4}/PE-CD_{8}, Caltag, USA). A total of 500 µl optilyse C was added, and the mixture was re-incubated at room temperature for 8 minutes. Two ml PBS was added after complete lysis of the red blood cells. After washing and centrifugation (1100 r/min), the samples were thoroughly mixed with 1 ml PBS and then analyzed by flow cytometer (FACSCalibur, B-D, USA).

**Statistical analysis**

Quantitative data were presented as mean±standard deviation (SD). Fisher exact probability test, homogeneity test, t test, two-way analysis of variance and least significant difference-t (LSD-t) analysis were used for statistical analysis on SPSS 13.0 software package. Differences were considered significant when P<0.05.

**RESULTS**

**Treatment and prognosis**

Clinical outcomes of the patients including complications and prognosis were recorded routinely (Table 2). Systemic inflammation response syndrome (SIRS), sepsis (SIRS with infection), and organ dysfunction were documented using established criteria.12-13

Of all 16 cases in the control group, 11 received emergency operations, 3 died of late multiple organ failure or sepsis, and 1 died of cerebral hernia. For the treatment group, emergency operations were performed...
in 10 patients and 3 died of multiple organ failure and sepsis in the later period. The incidence rates of sepsis and MODS in the control group were slightly higher than those in the treatment group. However, no significant differences were found in the complications and prognosis of the two groups ($P>0.05$).

**Variation of T-lymphocyte subpopulations in peripheral blood**

The T-lymphocyte subpopulations in peripheral blood of patients in two groups were detected, and the results were presented in Tables 3-5. On admission, the peripheral CD$_{4}^{+}$/CD$_{8}^{+}$ ratio in the two groups showed no significant differences, whereas the peripheral CD$_{4}^{+}$ T-lymphocytes and CD$_{4}^{+}$/CD$_{8}^{+}$ ratios in the two groups were all remarkably decreased on the 3rd day and 5th day after trauma ($P<0.01$). Meanwhile, the peripheral CD$_{8}^{+}$ T-lymphocyte showed no obvious change during the experimental period.

### Table 1. Comparison of gender, age, and ISS of patients between two groups ($\bar{x} \pm s$)

<table>
<thead>
<tr>
<th>Groups</th>
<th>$n$</th>
<th>Gender (male:female)</th>
<th>Age (year)</th>
<th>ISS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>16</td>
<td>12 : 4</td>
<td>32.88±10.99</td>
<td>31.25±10.85</td>
</tr>
<tr>
<td>Treatment group</td>
<td>17</td>
<td>11 : 6</td>
<td>34.88±11.45</td>
<td>29.88±8.62</td>
</tr>
<tr>
<td>$P$ values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Comparison of complications and prognosis between two groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>$n$</th>
<th>Survival</th>
<th>Sepsis (n,%)</th>
<th>MODS (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>16</td>
<td>Yes</td>
<td>No</td>
<td>Yes: No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 (75.0%)</td>
<td>12 (75.0%)</td>
<td>4 (25.0%): 7 (56.3%)</td>
</tr>
<tr>
<td>Treatment group</td>
<td>17</td>
<td>Yes</td>
<td>No</td>
<td>Yes: No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14 (82.6%)</td>
<td>10 (58.8%): 7 (41.2%): 8 (47.1%): 9 (52.9%)</td>
<td></td>
</tr>
<tr>
<td>$P$ values</td>
<td></td>
<td>0.688</td>
<td>0.465</td>
<td>0.732</td>
</tr>
</tbody>
</table>

*$P<0.01$, as compared with on admission; $^*$ $P<0.01$ as compared with the 3rd day after trauma.

### Table 3. Peripheral CD$_{4}^{+}$ T-lymphocytes changes of two groups ($\bar{x} \pm s$)

<table>
<thead>
<tr>
<th>Groups</th>
<th>$n$</th>
<th>On admission</th>
<th>3rd day</th>
<th>5th day</th>
<th>$F$ values</th>
<th>$P$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>16</td>
<td>45.09±6.57</td>
<td>32.40±6.02 *</td>
<td>34.19±5.63 *</td>
<td>39.149</td>
<td>0.000</td>
</tr>
<tr>
<td>Treatment group</td>
<td>17</td>
<td>46.10±6.02</td>
<td>34.30±5.46 *</td>
<td>38.55±6.47 *</td>
<td>34.917</td>
<td>0.000</td>
</tr>
<tr>
<td>$t$ values</td>
<td></td>
<td>0.460</td>
<td>0.951</td>
<td>2.061</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P$ values</td>
<td></td>
<td>0.649</td>
<td>0.349</td>
<td>0.048</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4. Peripheral CD$_{8}^{+}$ T-lymphocytes changes of two groups ($\bar{x} \pm s$)

<table>
<thead>
<tr>
<th>Groups</th>
<th>$n$</th>
<th>On admission</th>
<th>3rd day</th>
<th>5th day</th>
<th>$F$ values</th>
<th>$P$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>16</td>
<td>28.67±6.16</td>
<td>29.49±4.68</td>
<td>30.09±3.69</td>
<td>0.646</td>
<td>0.531</td>
</tr>
<tr>
<td>Treatment group</td>
<td>17</td>
<td>28.47±5.88</td>
<td>29.11±4.33</td>
<td>29.45±3.42</td>
<td>0.264</td>
<td>0.770</td>
</tr>
<tr>
<td>$t$ values</td>
<td></td>
<td>0.092</td>
<td>0.247</td>
<td>0.512</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P$ values</td>
<td></td>
<td>0.928</td>
<td>0.806</td>
<td>0.613</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 5. Peripheral CD$_{4}^{+}$/CD$_{8}^{+}$ ratio changes of two groups ($\bar{x} \pm s$)

<table>
<thead>
<tr>
<th>Groups</th>
<th>$n$</th>
<th>On admission</th>
<th>3rd day</th>
<th>5th day</th>
<th>$F$ values</th>
<th>$P$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>16</td>
<td>1.60±0.20</td>
<td>1.11±0.20 *</td>
<td>1.15±0.20 *</td>
<td>44.598</td>
<td>0.000</td>
</tr>
<tr>
<td>Treatment group</td>
<td>17</td>
<td>1.66±0.24</td>
<td>1.20±0.22 *</td>
<td>1.32±0.24 *</td>
<td>17.959</td>
<td>0.000</td>
</tr>
<tr>
<td>$t$ values</td>
<td></td>
<td>0.704</td>
<td>1.221</td>
<td>2.267</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P$ values</td>
<td></td>
<td>0.487</td>
<td>0.231</td>
<td>0.031</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* $P<0.01$, as compared with on admission.
On the 5th day after trauma, the peripheral CD\textsubscript{4}\textsuperscript{+} T-lymphocyte and CD\textsubscript{8}\textsuperscript{+} T-lymphocyte ratio in the treatment group renewed notably, showing statistically significant difference compared with the control group (\(P<0.05\)). Furthermore, compared with the 3rd day after trauma, the peripheral CD\textsubscript{8}\textsuperscript{+} T-lymphocyte in the treatment group was markedly increased on the 5th day, and the difference was considered significant (\(P<0.01\)).

**DISCUSSION**

The immuno-inflammatory response is dependent on intercellular communication through modification of cytokine response and fluctuation of peripheral immune cells such as natural killer (NK) cells, B cells, and T-lymphocyte subpopulations (CD\textsubscript{4}\textsuperscript{+} and CD\textsubscript{8}\textsuperscript{+} cells). It is well-known that T-lymphocytes express either the CD\textsubscript{8} or the CD\textsubscript{4} receptor on their surface. The CD\textsubscript{4}\textsuperscript{+} T-lymphocyte subpopulation is also called Help T cell (TH cell), and can be generally divided into three subpopulations of TH0, TH1, and TH2. TH0 cells are the original CD\textsubscript{4}\textsuperscript{+} T-lymphocytes, and can differentiate into the mature TH1 or TH2 cells by signal-activating in the microenvironment. TH1 cells mainly participate in promoting cell-mediated immune response, augmenting IgM and IgG2 synthesis by B cells, and activating macrophages. TH2 cells are concerned with promoting antibody-mediated immune response, leading to IgG1 and IgE responses, and increased numbers of local and/or circulating eosinophils. The CD\textsubscript{8}\textsuperscript{+} T-lymphocyte subpopulation can be divided into two subpopulations of suppressor T cell (TS cell) and cytotoxic T cell (TC cell). TS cells can quickly terminate or suppress immuno-inflammatory responses and TC cells can effectively kill the target through one or at least two distinct mechanisms. The CD\textsubscript{4}\textsuperscript{+} and CD\textsubscript{8}\textsuperscript{+} subpopulations collaborate and restrict each other, playing important modulation roles in the immuno-inflammatory reactions of the body to infection and injury. Consequently, the ratio of CD\textsubscript{4}/CD\textsubscript{8} cells is fairly constant among individuals, which can effectively reflect balanced condition between pro-inflammatory and anti-inflammatory processes.\(^{14}\)

Previous studies have showed that the immune function often disordered or maladjusted after the body suffered severe trauma, and it can be reflected on the signs of excessive inflammatory response in the early stage of trauma and subsequent suppression of adaptive immunity.\(^{13,15}\) This suppression, especially occurred in the T-lymphocyte mediated immune response, would obviously weaken the body immune function and incline the body to infection and organ failure, mainly presented as the peripheral CD\textsubscript{4}\textsuperscript{+} and CD\textsubscript{8}\textsuperscript{+} T-lymphocyte viability impairment, CD\textsubscript{4}/CD\textsubscript{8} ratio decrease, NK cell and lymphokine activated killer cell (LAK cell) viability reduction and so on. This sequence of events is thought to have very important clinical consequences since sepsis and MODS are the most frequent causes of death in patients who survive the first 24 hours.\(^{3,15}\) The results of this study also indicate the instant immuno-inflammatory regulation function disorder or inhibition in these patients, in view of the fact that peripheral CD\textsubscript{4}\textsuperscript{+} T-lymphocyte and CD\textsubscript{4}/CD\textsubscript{8} ratio markedly declined on the 3rd and 5th days after trauma. Consequently, balanced inflammatory responses are generally considered as a beneficial host response to injury.\(^{16}\)

Xuebijing is an intravenous injection consisting of 5 traditional Chinese medicines selected out from 36 traditional Chinese herb compound formulas. It is based on the “bacteria, endotoxin, inflammatory mediator treated simultaneously” theory proposed by Professor Wang Jinda.\(^{10}\) A series of animal experiments have fully demonstrated that Xuebijing injection can antagonise the disruptive effects of endotoxin, regulate body immune functions, decrease the stress-induced organ or tissue damage (liver, lung, endothelium of blood vessels, etc.), and notably improve the survive rate of animals with SIRS or MODS.\(^{7-11,18}\) Moreover, according to some clinical researches, early Xuebijing injection treatment showed favorable therapeutic effects and reliable safety on the patients with severe trauma, sepsis or MODS.\(^{17-20}\)

Our data from the present study showed that on the 5th day after trauma, the peripheral CD\textsubscript{4}\textsuperscript{+} T-lymphocyte and CD\textsubscript{4}/CD\textsubscript{8} ratio in the treatment group renewed notably, showing statistically significant difference compared with the control group. It indicates that for patients with severe trauma, an early use of Xuebijing injection can contribute to restoring the CD\textsubscript{4}/CD\textsubscript{8} ratio of peripheral blood, regulating the immune function, shortening the duration of disorder or suppression of T-lymphocyte subpopulations regulating network, and thereby promoting a more balanced profile of immunologic function.

In summary, we found that the immuno-inflamma-
tory regulating function would be disordered or suppressed after the body suffered severe trauma, and an early use of Xuebijing injection was effective in correcting disorder or suppression of T-lymphocyte subpopulations regulating network and promoting a balanced profile of immunologic function, which may be one of the protective mechanisms of Xuebijing injection. This study also provided a new viewpoint for the development of traditional Chinese herb to treat patients with severe injury. However, as for the molecular mechanism and concrete signal transduction process in Xuebijing regulating network, they still need further study.

REFERENCES


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