CLINICAL EXPERIENCE

Influence of TCM Therapy for Supplementing Pi (脾) and Nourishing Shen (肾) on Dendritic Cell Function in Patients with Chronic Hepatitis B Treated by Lamivudine*

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ABSTRACT  Objective: To observe the influence of traditional Chinese medicine (TCM) therapy for supplementing Pi (脾) and nourishing Shen (肾, SPNS) on dendritic cell function in patients with chronic hepatitis B (CHB) treated by lamivudine. Methods: Sixty CHB patients with positive HBeAg were equally randomized by digital table into two groups: the observation group and the control group. Patients in the control group were treated with lamivudine only, while patients in the observation group were treated with lamivudine combined with SPNS formula, for 12 weeks. The phenotype and function of dendritic cell, as well as its secretion factor interleukin 12 (IL-12) in all patients were determined after termination of therapy and the impacts on alanine transaminase (ALT) and HBV-DNA were observed. Results: The phenotypes of dendritic cells such as CD1a, CD80, CD86, human leukocyte antigen (HLA-DR) and intercellular adhesion molecule-1, as well as the levels of stimulation index (SI) and IL-12 were higher in the observation group than those in the control group (P<0.05 or P<0.01). Meanwhile, significant difference between the two groups was also shown in the normalizing rates of ALT and HBV-DNA (P<0.05). Conclusion: TCM therapy for SPNS can significantly improve the function of dendritic cells in patients with CHB treated by lamivudine and enhance the early stage response of patients to the treatment.

KEY WORDS  TCM therapy for supplementing Pi and nourishing Shen, lamivudine, chronic hepatitis B, dendritic cells

Dendritic cells (DC) are currently known as the antigen presenting cells with the most potent function, and its function, normal or abnormal, is directly correlated with the virtual specific immune response of the organism. In patients with chronic hepatitis B (CHB), the existence of DC antigen presentation abnormality has been approved, and recognized as an important impacting factor for patients' prognosis(1). In the recent years, the authors, through the use of Chinese drugs for supplementing Pi (脾) and nourishing Shen (肾, SPNS) in treating CHB coordinated with lamivudine, found that these Chinese drugs have an apparent regulatory effect on patients' DC antigen presenting function, which is reported in the following text.

METHODS

Clinical Data
The sixty subjects enrolled were out-patients who visited, from June, 2006 to February, 2007, the authors' Hospital, and whose diagnosis of CHB matched the diagnostic standard and indication of anti-viral treatment listed in the "Guidance for Prevention and Treatment of Chronic Hepatitis B"(2) issued by Chinese Society of Liver Disease and Chinese Society of Infectious Diseases, Chinese Medical Association, 2005. They were assigned to two groups by a randomizing digital table, with 30 in each group. The 30 patients in the observation group were 18 males and 12 females, aged 21-51 years, 37.20 ± 8.84 years on average, with course of diseases at 5.90 ± 2.51 years. The 30 patients in the control group were 16 males and 14 females, aged 26-49 years, 38.47 ± 11.99 years on average, with course of diseases at 5.93 ± 3.01 years. Comparison between the two groups shows statistically insignificant difference in sex, age and course of disease (P>0.05). All the subjects never received anti-viral drugs like lamivudine or immune regulatory treatment before. Those complicated viral infections of HAV, HCV and HEV, as well as complications of fatty liver, hereditary metabolic liver and immune related diseases were excluded.

Treatment
To all patients, lamivudine (trade name Heptovir,
a product of Glaxo-Wellcome Company, batch number 06050029) 100 mg was administered once every day. To the patients in the observation group, Chinese drugs for SPNS were given coordinately with the prescription containing milkvetch root, human placenta, aweto, grossy privet fruit and notoginseng, in the proportion of 3:1:2:1:2 in terms of weight. The remedy was prepared into tablet form by the Chinese Crude Drug Factory of the Zhejiang Chinese Medical University. Each tablet contains 1.5 g of crude drugs, and were administered 3 times every day, at 4 tablets each time. The therapeutic course was 12 weeks for both groups. All drugs for liver protection and lowering blood transaminase were withdrawn in the observation period.

Items and Methods of Observation
The expressions of DC phenotype, CD1a, CD80, CD86, human leukocyte antigen DR (HLA-DR) and intercellular adhesion molecule-1 (ICAM-1), as well as its functional antigen presenting function (MLR) and secretion factor interleukin-12 (IL-12) were determined at the end of the 12-week treatment. The levels of alanine transaminase (ALT), HBV-DNA and 3 serial tests for CHB were monitored every 4 weeks during the observation period.

Instrument and reagents used for determining above-mentioned indices were: A HITACHI fully-automatic biochemical analyzer type 7180 (Japan) used for ALT. The reagents were a product of the Langdo Co. (UK), batch No. AL0623. For the 3 serial tests of LAB enzyme labeling apparatus type MKⅢ, ELISA was purchased for use from the Beijing Kewei Co., Ltd. of Reagents, batch No. 20060607. For the HBV-DNA, RT-PCR was used with the test kit provided by the Da'an Gene Co., Ltd. of Sun Yat-sen University of Medical Sciences, batch No. 2006012. For the DC phenotype, a Beckman-Coulter EPICS XLTM flow cytometer was used with the outcome expressed by the percentage of positive cells. For the DC MLR, the MTT method was used at 570 nm and expressed by OD value, and the DC stimulation index (SI) was calculated by the formula of SI=At 570 at experimental pore/At 570 at control pore. For IL-12, a BIO-TEK enzyme labeling apparatus (USA) was used and the test conducted according to the instruction book provided by the BD Company (USA). The source and culture of DC cells were performed in reference of the Romani method7.

Statistical Analysis
Data were expressed by x±s, managed by the SPSS 13.0 software package, with a t-test adopted for independent samples.

RESULTS

Comparison of DC Phenotypes
The DC phenotypes CD1a, CD80, CD86, HLA-DR and ICAM-1 after treatment were higher in the observation group than those in the control group with statistical significance (P<0.05 or P<0.01, Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Case</th>
<th>CD1a</th>
<th>CD80</th>
<th>CD86</th>
<th>HLA-DR</th>
<th>ICAM-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>30</td>
<td>31.76±5.46</td>
<td>46.96±3.82</td>
<td>41.78±4.38</td>
<td>37.49±2.59</td>
<td>32.92±4.99</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>28.47±6.09</td>
<td>44.81±3.73</td>
<td>38.64±6.22</td>
<td>35.86±3.60</td>
<td>29.20±4.75</td>
</tr>
</tbody>
</table>

Note: *P<0.05, **P<0.01, compared with the control group

Comparison of DC Function and Secretion Factor IL-12
After treatment, the levels of SI and IL-12 in the observation group were evidently higher than those in the control group, showing statistical significance (P<0.05, Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>Case</th>
<th>SI</th>
<th>IL-12 (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>30</td>
<td>0.50±0.06*</td>
<td>73.39±4.88*</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>0.47±0.05</td>
<td>70.75±4.71</td>
</tr>
</tbody>
</table>

Note: *P<0.05, compared with the control group

Comparison of Normalizing Rates of ALT and HBV-DNA
As shown in Table 3, the normalizing rates of ALT and HBV-DNA in the observation group were markedly higher than those in the control group, showing statistical difference between them (P<0.05).

<table>
<thead>
<tr>
<th>Group</th>
<th>Case</th>
<th>Normalizing rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>30</td>
<td>80.0* 73.0</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>57.0  50.0</td>
</tr>
</tbody>
</table>

Note: *P<0.05, compared with the control group

DISCUSSION
An epoch-making advance has been achieved in the treatment of CHB due to the emergence of nucleotide-like drugs, but they are not effective for all CHB patients, especially with disadvantages such as a low full responsive rate, no end-point course, liability to recurrence after withdrawal and virus variation after