Effect of Thymosin Injection combined with entecavir therapy on the viral load, liver fibrosis and immune response of patients with compensated liver cirrhosis

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ABSTRACT

Objective: To study the effect of Thymosin Injection combined with entecavir therapy on the viral load, liver fibrosis and immune response of patients with compensated liver cirrhosis.

Methods: Patients who were diagnosed with chronic hepatitis b complicated by compensated liver cirrhosis in our hospital between March 2015 and January 2017 were selected and randomly divided into two groups, observation group received Thymosin Injection combined with entecavir therapy, and control group received entecavir therapy. The viral load, liver function injury indexes, liver fibrosis indexes, immune cytokines and immune cell apoptosis molecules were measured 24 weeks after treatment. Results: 24 weeks after treatment, HBV-DNA copy number in peripheral blood as well as ALT, AST, TBIL, HA, PC-III, LN, C-IV, IL-4, IL-10, TGF-β1, IL-17 and IL-22 levels in serum of observation group was significantly lower than those of control group, and peripheral blood FAS, CYCS, CASP8, CASP9 and CASP3 mRNA expression were significantly lower than those of control group. Conclusion: Thymosin Injection combined with entecavir therapy can reduce viral load, improve liver function, inhibit liver fibrosis and regulate immune function in patients with compensated liver cirrhosis.

1. Introduction

Chronic hepatitis B is a clinical common infectious disease of digestive system. Hepatitis b virus infection will not only cause liver dysfunction, but also increase the risk of cirrhosis. Epidemiological data show that approximately 25% of patients with hepatitis b virus infection will eventually develop cirrhosis[1,2]. Persistent infection and high-level replication of hepatitis b virus is an important factor causing the occurrence and progression of liver cirrhosis and antiviral therapy is the core link in treating chronic hepatitis b and preventing or delaying cirrhosis of the liver. Entecavir is a common antiviral drug for clinical treatment of chronic hepatitis b, it can effectively inhibit viral replication, but the efficacy of monotherapy is not ideal for chronic hepatitis b that has developed cirrhosis[3]. The thymosin is an artificially synthesized polypeptide with immunomodulatory effect, which can not only enhance the antiviral immune response of the body, but also delay the course of liver fibrosis[4]. In the following studies, we analyzed the effect of Thymosin Injection combined with entecavir on the viral load, liver fibrosis and immune response of patients with compensated liver cirrhosis.

2. Case information and research methods

2.1 General case information

Patients who were diagnosed with chronic hepatitis b complicated by compensated liver cirrhosis in our hospital between March 2015 and January 2017 were selected as the research subjects, all patients were in accordance with the diagnostic criteria for chronic hepatitis b and compensated liver cirrhosis, and HBV-DNA quantitative was > 1 × 10^5 copies/L. Patients with autoimmune liver disease, alcoholic liver disease and other hepatitis virus infections were excluded. A total of 84 patients were enrolled and divided into two groups by random number table, each with 42 cases. Observation group included 25 male cases and 17 female cases that were 36-
56 years old, and with HBV-DNA quantitative $13.9 \times 10^3$ copies/L; control group included 27 male cases and 15 female cases that were 34-58 years old, and with HBV-DNA quantitative $14.2 \times 10^3$ copies/L. There was no statistically significant difference in general information between the two groups ($P>0.05$).

### 2.2 Therapy

Both groups of patients received entecavir antiviral therapy as follows: entecavir tablets, taken orally, 0.5 mg/time, 1 time/d. Based on entecavir antiviral therapy, observation group received thymosin therapy, which was as follows: Thymosin 1 for Injection, subcutaneous injection, 1.6 mg/time, 1 time/day. Both groups were treated for 24 weeks in a row.

### 2.3 Serum index detecting

24 weeks after treatment, 3-5 mL of fasting cubital venous blood was collected and centrifuged to get serum, automatic biochemical analyzer was used to detect the contents of ALT, AST and TBIL, and enzyme-linked immunosorbent assay kit was used to detect the contents of HA, PC-III, LN, C-IV, IL-4, IL-10, TGF-β1, IL-17 and IL-22.

### 2.4 Peripheral blood index detecting

24 weeks after treatment, 2 mL of fasting cubital venous blood was collected and divided into two parts. One was used to extract whole blood DNA, and fluorescence quantitative PCR amplification was conducted to determine HBV-DNA quantitative; the other was used to extract whole blood RNA and synthesize it into cDNA by reverse transcription, then fluorescence quantitative PCR amplification was conducted to determine the mRNA expression of FAS, CYCS, CASP8, CASP9 and CASP3.

### 2.5 Statistical methods

SPSS 21.0 software was used to input data, the differences in measurement data between two groups were by test and $P<0.05$ indicated statistical significance in differences in test results.

### 3. Results

#### 3.1 Viral load and liver function injury indexes

24 weeks after treatment, analysis of viral load ($\times 10^3$ copies/L) as well as liver function injury indexes ALT (U/L), AST (U/L) and TBIL (μmol/L) between two groups of patients was as follows: HBV-DNA copy number in peripheral blood as well as ALT, AST and TBIL levels in serum of observation group was significantly lower than those of control group. Differences in HBV-DNA copy number in peripheral blood as well as ALT, AST and TBIL levels in serum were statistically significant between two groups of patients 24 weeks after treatment ($P<0.05$).

#### 3.2 Liver fibrosis index levels

24 weeks after treatment, analysis of serum liver fibrosis indexes HA, PC-III, LN and C-IV levels between two groups of patients was as follows: HA, PC-III, LN and C-IV levels in serum of observation group were significantly lower than those of control group. Differences in HA, PC-III, LN and C-IV levels in serum were statistically significant between two groups of patients 24 weeks after treatment ($P<0.05$).

#### 3.3 Serum immune cytokine levels

24 weeks after treatment, analysis of serum immune cytokines IL-4 (pg/mL), IL-10 (pg/mL), TGF-β1 (ng/mL), IL-17 (ng/mL) and IL-22 (pg/mL) levels between two groups of patients was as follows: IL-4, IL-10, TGF-β1, IL-17 and IL-22 levels in serum of observation group were significantly lower than those of control group. Differences in IL-4, IL-10, TGF-β1, IL-17 and IL-22 levels in serum were statistically significant between two groups of patients 24 weeks after treatment ($P<0.05$).

### Table 1.

Comparison of viral load and liver function injury indexes.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>HBV-DNA</th>
<th>ALT</th>
<th>AST</th>
<th>TBIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>42</td>
<td>3.48±0.52</td>
<td>34.52±5.54</td>
<td>31.32±4.52</td>
<td>15.21±1.80</td>
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<tr>
<td>Control group</td>
<td>42</td>
<td>7.71±0.90</td>
<td>49.39±6.27</td>
<td>47.24±5.95</td>
<td>21.35±2.85</td>
</tr>
<tr>
<td>$t$</td>
<td></td>
<td>12.181</td>
<td>7.694</td>
<td>7.283</td>
<td>8.283</td>
</tr>
<tr>
<td>$P$</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

### Table 2.

Comparison of serum liver fibrosis indexes (g/L).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>HA</th>
<th>PC-III</th>
<th>LN</th>
<th>C-IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>42</td>
<td>313.52±55.64</td>
<td>103.41±13.85</td>
<td>127.68±15.88</td>
<td>93.84±11.38</td>
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<tr>
<td>Control group</td>
<td>42</td>
<td>434.51±55.94</td>
<td>167.64±20.34</td>
<td>189.41±21.36</td>
<td>142.48±17.95</td>
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<tr>
<td>$t$</td>
<td></td>
<td>7.598</td>
<td>8.482</td>
<td>7.182</td>
<td>8.239</td>
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<tr>
<td>$P$</td>
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<td>&lt;0.05</td>
<td>&lt;0.05</td>
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</tr>
</tbody>
</table>
Comparison of peripheral blood immune cell apoptosis molecule expression.

Table 4.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>FAS</th>
<th>CYCS</th>
<th>CASP8</th>
<th>CASP9</th>
<th>CASP3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>42</td>
<td>0.38±0.06</td>
<td>0.29±0.04</td>
<td>0.21±0.05</td>
<td>0.45±0.07</td>
<td>0.42±0.06</td>
</tr>
<tr>
<td>Control group</td>
<td>42</td>
<td>1.02±0.15</td>
<td>1.04±0.16</td>
<td>0.98±0.11</td>
<td>1.05±0.14</td>
<td>1.07±0.11</td>
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<td>t</td>
<td></td>
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<td>&lt;0.05</td>
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<tr>
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<td></td>
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</tbody>
</table>

3.4 Peripheral blood immune cell apoptosis molecule expression

24 weeks after treatment, analysis of peripheral blood immune cell apoptosis molecules FAS, CYCS, CASP8, CASP9 and CASP3 expression between two groups of patients was as follows: peripheral blood FAS, CYCS, CASP8, CASP9 and CASP3 mRNA expression of observation group were significantly lower than those of control group. Differences in peripheral blood FAS, CYCS, CASP8, CASP9 and CASP3 mRNA expression were statistically significant between two groups of patients 24 weeks after treatment (P<0.05).

4. Discussion

Cirrhosis is the final stage of chronic hepatitis b, and liver fibrosis is a prominent pathological feature of patients with cirrhosis. In the progress of cirrhosis of the liver, continuous hepatitis b virus replication can cause damage to liver cells, which will on the one hand, cause inflammation reaction activation, on the other hand, cause fibrous connective tissue hyperplasia repair, and finally result in the occurrence of liver fibrosis[5,6]. Antivirus immunocompromise is closely related to the uncleanable hepatitis b virus and its continuous replication, and immunomodulation drugs have been increasingly used in recent years to treat hepatitis b cirrhosis. Thymosin 1 is artificial immunocompetent polypeptide that can promote the proliferation and differentiation of T cells, B cells, NK cells and other immune cells, and increase the synthesis and secretion of a variety of immunocompetent molecules so as to help remove the hepatitis B virus[7,8]. Continuous hepatitis b virus replication and large viral load are the important pathological factors causing liver damage and hepatic fibrosis, the viral load and liver damage indexes were analyzed in the study to reflect the effect of thymosin therapy on hepatitis b virus load, and the results showed that HBV-DNA copy number in peripheral blood as well as ALT, AST and TBIL levels in serum of observation group was significantly lower than those of control group. This shows that thymosin combined with entecavir treatment can be more effective than entecavir monotherapy in inhibiting the hepatitis b virus replication and reducing the virus load, the viral load is lower and liver function damage is lighter after treatment.

In the process of liver fibrosis formation, the synthesis of collagen, protein, polysaccharide, glycoprotein and other compositions in extracellular matrix increases, and their degradation decreases, which will cause the extracellular matrix to deposit excessively in the liver and form local tissue fibrosis. HA is a type of macromolecular glucosamine polysaccharide in the extracellular matrix that is mainly synthesized and secreted by the hepatic mesenchymal cells[9], LN is a glycoprotein in the extracellular matrix that is mainly distributed in the blood vessel walls and bile duct walls, and liver cell damage will affect the catabolism of HA and LN, make the HA and LN accumulate in liver tissue and cause fibrosis[10]; PC-III is the precursor of type III collagen. The deposition of extracellular matrix and the deposition of collagen in liver tissues are most significant during hepatic fibrosis process, and the content of PC-III can reflect the collagen anabolism[11,12]; C-IV is the main collagen component in the basement membrane of the cell, and the remodeling of the basement membrane during the liver fibrosis process is accompanied by the increased synthesis of C-IV[13]. In this study, the changes of extracellular matrix metabolism indexes were analyzed to reflect the thymosin treatment effect on the liver fibrosis process, and the result showed that HA, PC-III, LN and C-IV levels in serum of observation group were significantly lower than those of control group. This indicates that thymosin combined with entecavir treatment can be more effective than entecavir monotherapy in inhibiting the process of hepatic fibrosis in patients with cirrhosis.

Disorders of immune response and inflammatory response in the continuous hepatitis b virus infection can lead to chronic liver injury and fibrosis. CD4+T lymphocytes are immune cells that play an important role in liver inflammation, injury and fibrosis. The IL-4, IL-10 and TGF-β1 secreted by Treg subsets participate in the regulation of antiviral immune response and liver fibrosis at the same time, IL-4 and IL-10 are with negative immunomodulatory activity and can inhibit the antiviral immune response to cause the immune escape and continuous replication of virus, and TGF-β1 can accelerate the extracellular matrix secretion, which can accelerate the process of liver fibrosis[14]. The IL-17 secreted by Th17 subset
has significant pro-inflammatory activity, which can stimulate fibroblast activation and promote local infiltration of inflammatory cells to cause local tissue fibrosis[15]. The IL-22 secreted by Th22 subset can be combined with the IL-22R on the surface of the hepatic stellate cells to activate the STAT3 signaling pathway and then cause liver tissue fibrosis[16]. In this study, the levels of immune cytokines were analyzed to reflect the thymosin treatment effect on the thymosin treatment effect on the immune response, and the results showed that IL-4, IL-10, TGF-β1, IL-17 and IL-22 levels in serum of observation group were significantly lower than those of control group. This shows that the thymosin combined with entecavir therapy may be more effective than entecavir monotherapy in improving the immune response in patients with cirrhosis.

Immune response disorder is an important factor that causes continuous virus replication in patients with hepatitis B cirrhosis. Excessive immune cell apoptosis can cause immune cell function to change and result in immune response disorders. The death ligand pathway and mitochondria-dependent pathway are the exogenous pathway and endogenous pathway that regulate the apoptosis of immune cells respectively[17]. FAS is the membrane receptor mediating death ligand pathway, and it can induce apoptosis by CASP8 activation[18]; that the CYCS enters in the mitochondria is the necessary step for the mitochondrial apoptosis pathway, and the CYCS in the CYCS can induce apoptosis via CASP9 activation[19]. CASP3 is a common downstream molecule of exogenous apoptosis and endogenous apoptosis, which can directly execute apoptosis. In the study, analysis of immune cell apoptosis molecule expression in peripheral blood showed that peripheral blood FAS, CYCS, CASP8, CASP9 and CASP3 mRNA expression of observation group were significantly lower than those of control group. This indicates that the thymosin combined with entecavir therapy is more effective than entecavir monotherapy in inhibiting the apoptosis of immune cells in patients with cirrhosis.

Thymosin injection combined with entecavir can be more effective than entecavir monotherapy in reducing viral load, reducing liver function injury, delaying liver fibrosis process, suppressing immune cell apoptosis and regulating immune response in patients with compensated liver cirrhosis.

References