Effect of Dahuang Zhechong pill combined with antiviral therapy on serum virus replication indexes as well as immunity and inflammation indexes in patients with chronic hepatitis B cirrhosis

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ABSTRACT

Objective: To study the effect of Dahuang Zhechong pill combined with antiviral therapy on serum virus replication indexes as well as immunity and inflammation indexes in patients with chronic hepatitis B cirrhosis. Methods: A total of 104 patients with chronic hepatitis B cirrhosis who were treated in our hospital between May 2013 and April 2016 were selected and randomly divided into combined treatment group and western medicine treatment group who accepted Dahuang Zhechong pill combined with entecavir treatment and entecavir monotherapy respectively. Before and after treatment, the HBV-DNA loads, liver fibrosis index and immune cell levels as well as inflammatory cytokine levels were determined. Results: HBV-DNA loads of both groups of patients at 48 weeks after treatment were significantly lower than those before treatment, and the HBV-DNA loads were not significantly different between the two groups after treatment; serum HA, LN, PC-III, IV-C, IL-15, IL-16 and TGF-β1 levels as well as peripheral blood Treg cell levels of both groups at 24 weeks and 48 weeks after treatment were significantly lower than those before treatment while peripheral blood NK cell levels were higher than those before treatment, and serum HA, LN, PC-III, IV-C, IL-15, IL-16 and TGF-β1 levels as well as peripheral blood Treg cell levels of combined treatment group were significantly lower than those of western medicine treatment group while peripheral blood NK cell levels were higher than those of western medicine treatment group. Conclusion: Dahuang Zhechong pill combined with antiviral treatment of chronic hepatitis B cirrhosis can regulate the immune response and inflammatory reaction without affecting the inhibiting effect of antiviral drugs on viral replication.

1. Introduction

Chronic hepatitis b is an important cause of cirrhosis of the liver, and about 6%-20% of the patients with chronic hepatitis b virus may develop cirrhosis of the liver. In the pathological process of liver cirrhosis, the hepatitis b virus infection-induced immune response and inflammatory response disorders as well as abnormal cytokine secretion are the important pathological links causing liver fibrosis[1,2], and regulating immune response and inflammatory response is the important therapeutic target to slow down the pathological process of liver cirrhosis[3]. Antiviral drugs are the main ways for clinical treatment of viral hepatitis, which can not only inhibit viral replication, but also delay the pathological process of liver fibrosis. But antiviral drugs alone cannot completely block the occurrence and development of liver fibrosis, and anti-fibrosis drugs are required to reverse the process of liver fibrosis and delay the development of liver cirrhosis. Dahuang Zhechong pill is a Chinese patent medicine that has been used in the treatment of viral hepatitis in recent years, and it can target the TCM pathogenesis of liver fibrosis to exert the effects of promoting blood circulation to remove blood stasis as well as clearing away heat and toxic materials. In the following study, the effect of Dahuang Zhechong pill combined with antiviral therapy on serum virus replication indexes as well as immunity and inflammation indexes in patients with chronic hepatitis B cirrhosis was analyzed.
2. Subjects and methods

2.1 Research subjects

A total of 104 patients with chronic hepatitis B cirrhosis who were treated in our hospital between May 2013 and April 2016 were selected as the research subjects, all the patients were in accordance with the diagnosis of chronic hepatitis B cirrhosis, they were HBeAg positive and with HBV DNA quantification $\geq 2.00 \times 10^{7}$ IU/mL, and the liver cirrhosis was confirmed by abdominal ultrasound or abdominal CT; patients with the liver cirrhosis caused by alcohol, drugs, cholestasis and autoimmune hepatitis, and associated with other types of hepatitis B virus infection were ruled out, patients associated with autoimmune diseases and those allergic to entecavir and Dahuang Zhechong pill were excluded. Random number table was used to divide the included patients into combined treatment group and western medicine treatment group, 52 cases in each group. Combined treatment group accepted Dahuang Zhechong pill combined with entecavir treatment, including 29 male cases and 23 female cases that were 42-65 years old; western medicine treatment group received entecavir treatment, including 29 male cases and 23 female cases that were 42-65 years old; western medicine treatment group, 52 cases in each group, and comparison between groups after treatment showed significant differences ($P<0.05$); serum HA, LN, PC-III and IV-C levels of both groups were not significantly different between the two groups after treatment ($P>0.05$).

2.2 Treatment methods

Western medicine treatment group received entecavir treatment, and the method was as follows: entecavir tablets 0.5 mg, taken orally, 1 time/d, for continuous 48 weeks of treatment; combined treatment group received Dahuang Zhechong pill combined with entecavir treatment, and the method was as follows: entecavir tablets 0.5 mg, taken orally, 1 time/d; Dahuang Zhechong pill, 4 pills, taken orally, 2 times/d, for continuous 48 weeks of treatment.

2.3 HBV–DNA replication determination methods

48 weeks after treatment, 3 mL of peripheral blood was collected from two groups of patients, and the HBV-DNA fluorescence quantitative PCR detection kits provided by Daan gene diagnose center of Sun Yat-sen University School of Medicine were used to determine HBV-DNA loads in peripheral blood samples.

2.4 Peripheral blood index and serum index detection methods

Before treatment as well as 24 weeks and 48 weeks after treatment, 5 mL of peripheral blood was collected from two groups of patients and centrifuged to separate serum, and enzyme-linked immunosorbert assay kits were used to determine hyaluronic acid (HA), laminin (LN), procollagen type III (PC-III), type IV collagen (IV-C), interleukin-15 (IL-15), IL-16 and transforming growth factor-$\beta_1$ (TGF-$\beta_1$) contents; another $5\text{ml}$ of peripheral blood was collected and directly used for the incubation of CD3, CD4, CD25, CD45, CD56 and Foxp3 fluorescent antibody, and then flow cytometer was used to determine Treg cell (CD4$^{-}$CD25$^{+}$Foxp3$^{-}$/CD4$^{-}$) and NK cell (CD3/CD56/CD45$^{-}$) contents.

2.5 Statistical methods

SPSS 17.0 software was used to input HBV-DNA load, peripheral blood indexes and serum indexes, differences in above data between two groups was by t test and $P<0.05$ indicated statistical significance in differences.

3. Results

3.1 HBV–DNA loads of two groups of patients

Before treatment and 48 weeks after treatment, HBV-DNA loads of combined treatment group were $(6.67\pm 0.89) \times 10^7$ IU/mL and $(0.57 \pm 0.07) \times 10^7$ IU/mL respectively; HBV-DNA loads of western medicine treatment group were $(6.82 \pm 0.94) \times 10^7$ IU/mL and $(0.54 \pm 0.07) \times 10^7$ IU/mL respectively. After t test, HBV-DNA loads of both groups of patients after treatment were significantly lower than those before treatment ($P<0.05$), and the HBV-DNA loads were not significantly different between the two groups after treatment ($P>0.05$).

3.2 Serum liver fibrosis index levels of two groups of patients

Before treatment as well as 24 weeks and 48 weeks after treatment, analysis of serum liver fibrosis indexes HA (ng/mL), LN (ng/mL), PC-III (μg/mL) and IV-C (μg/mL) levels between two groups of patients was as follows: 24 weeks and 48 weeks after treatment, serum HA, LN, PC-III and IV-C levels of both groups were significantly lower than those before treatment, and comparison within group before and after treatment showed significant differences ($P<0.05$); serum HA, LN, PC-III and IV-C levels of combined treatment group 24 weeks and 48 weeks after treatment were significantly lower than those of western medicine treatment group, and comparison between groups after treatment showed differences.

4. Discussion

The results show that Dahuang Zhechong pill and entecavir treatment for patients with chronic hepatitis B cirrhosis can significantly reduce HBV-DNA loads, HBV-DNA replication, the levels of liver fibrosis indexes, and CD4$^+$ Treg cell count, and CD8$^+$ NK cell count, and thus significantly improve the T-regulatory function of the T lymphocytes. The study also shows that Dahuang Zhechong pill and entecavir treatment for patients with chronic hepatitis B cirrhosis can significantly reduce the levels of liver fibrosis indexes, and improve the T-regulatory function of the T lymphocytes.

Table 1.

Comparison of serum liver fibrosis indexes between two groups of patients before and after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>$n$</th>
<th>Time point</th>
<th>HA</th>
<th>LB</th>
<th>PC-III</th>
<th>IV-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined treatment group</td>
<td>52</td>
<td>Before treatment</td>
<td>156.5±22.4</td>
<td>49.1±6.4</td>
<td>47.6±7.8</td>
<td>54.5±7.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24 weeks after treatment</td>
<td>77.3±8.2$^{*}$</td>
<td>20.3±3.6$^{*}$</td>
<td>23.1±3.9$^{*}$</td>
<td>27.1±4.6$^{*}$</td>
</tr>
<tr>
<td></td>
<td>48 weeks after treatment</td>
<td>48.1±6.7$^{*}$</td>
<td>16.6±2.7$^{*}$</td>
<td>14.8±2.8$^{*}$</td>
<td>18.9±2.7$^{*}$</td>
<td></td>
</tr>
<tr>
<td>Western medicine treatment group</td>
<td>52</td>
<td>Before treatment</td>
<td>159.1±19.4</td>
<td>48.7±7.8</td>
<td>48.6±7.4</td>
<td>55.5±8.1</td>
</tr>
<tr>
<td></td>
<td>24 weeks after treatment</td>
<td>103.5±12.8$^{*}$</td>
<td>32.8±4.7$^{*}$</td>
<td>32.9±5.7$^{*}$</td>
<td>32.4±5.5$^{*}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48 weeks after treatment</td>
<td>88.4±10.3$^{*}$</td>
<td>23.5±3.9$^{*}$</td>
<td>20.3±4.1$^{*}$</td>
<td>23.1±4.1$^{*}$</td>
<td></td>
</tr>
</tbody>
</table>

$^{*}$: comparison within group before and after treatment, $P<0.05$; $^*$: comparison between groups after treatment, $P<0.05$.  

* * *
significant differences (P<0.05).

3.3 Peripheral blood immune cell levels of two groups of patients

Before treatment as well as 24 weeks and 48 weeks after treatment, analysis of peripheral blood immune cells NK cell and Treg cell levels between two groups of patients was as follows: 24 weeks and 48 weeks after treatment, peripheral blood NK cell levels of both groups were significantly higher than those before treatment while Treg cell levels were significantly lower than those before treatment, and comparison within group before and after treatment showed significant differences (P<0.05); peripheral blood NK cell levels of combined treatment group 24 weeks and 48 weeks after treatment were significantly higher than those of western medicine treatment group while Treg cell levels were significantly lower than those of western medicine treatment group, and comparison between groups after treatment showed significant differences (P<0.05).

Table 2.
Comparison of peripheral blood immune cell levels between two groups of patients before and after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time point</th>
<th>NK cell</th>
<th>Treg cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined treatment group</td>
<td>52</td>
<td>Before treatment</td>
<td>12.51±1.85</td>
<td>8.61±1.02</td>
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<tr>
<td></td>
<td></td>
<td>24 weeks after</td>
<td>18.75±2.86*</td>
<td>4.62±0.85*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>treatment</td>
<td>20.33±3.27*</td>
<td>3.98±0.67*</td>
</tr>
<tr>
<td>Western medicine</td>
<td>52</td>
<td>Before treatment</td>
<td>12.71±1.44</td>
<td>8.89±0.97</td>
</tr>
<tr>
<td>treatment group</td>
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<td>24 weeks after</td>
<td>15.02±1.78*</td>
<td>6.72±0.84*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>treatment</td>
<td>16.85±2.38</td>
<td>5.89±0.77*</td>
</tr>
</tbody>
</table>

*: comparison within group before and after treatment, P<0.05; &: comparison between groups after treatment, P<0.05.

3.4 Serum cytokine levels of two groups of patients

Before treatment as well as 24 weeks and 48 weeks after treatment, analysis of serum cytokines IL-15, IL-16 and TGF-β1 levels between two groups of patients was as follows: 24 weeks and 48 weeks after treatment, serum IL-15, IL-16 and TGF-β1 levels of both groups were lower than those before treatment, and comparison within group before and after treatment showed significant differences (P<0.05); serum IL-15, IL-16 and TGF-β1 levels of combined treatment group 24 weeks and 48 weeks after treatment were lower than those of western medicine treatment group, and comparison between groups after treatment showed significant differences (P<0.05).

Table 3.
Comparison of serum cytokine levels between two groups of patients before and after treatment (ng/mL).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time point</th>
<th>IL-15</th>
<th>IL-16</th>
<th>TGF-β1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined treatment group</td>
<td>52</td>
<td>Before treatment</td>
<td>231.5±35.2</td>
<td>106.8±16.5</td>
<td>495.6±61.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24 weeks after</td>
<td>142.4±17.8*</td>
<td>47.6±7.2*</td>
<td>257.6±35.7*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>treatment</td>
<td>118.7±13.7*</td>
<td>33.4±4.9*</td>
<td>201.2±29.5*</td>
</tr>
<tr>
<td>Western medicine</td>
<td>52</td>
<td>Before treatment</td>
<td>233.1±37.6</td>
<td>108.3±17.6</td>
<td>497.1±67.4</td>
</tr>
<tr>
<td>treatment group</td>
<td></td>
<td>24 weeks after</td>
<td>189.2±26.8*</td>
<td>78.4±9.3*</td>
<td>331.4±46.7*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>treatment</td>
<td>164.3±17.8*</td>
<td>52.9±7.5*</td>
<td>275.1±41.9*</td>
</tr>
</tbody>
</table>

*: comparison within group before and after treatment, P<0.05; &: comparison between groups after treatment, P<0.05.

4. Discussion

Chronic hepatitis b is the important cause of cirrhosis, and the hepatitis b virus replication-induced immune response and inflammatory response disorder as well as abnormal secretion of cytokines is the important pathological link causing liver fibrosis[4]. Antiviral western medicine drugs can effectively inhibit viral replication and delay the pathological process of liver fibrosis to a certain extent, but cannot completely reverse the process of liver cirrhosis and increase the risk of liver cancer after liver cirrhosis[5,6]. Dahuang Zhechong pill is the Chinese patent drug that has been used for treatment of chronic hepatitis b drugs in recent years, and the existing domestic research has shown that its combination with antiviral western medicine drugs can delay the development of liver cirrhosis in patients with chronic hepatitis b[7]. In the study, in order to define the effect of Dahuang Zhechong pill for treatment of chronic hepatitis b cirrhosis, HBV-DNA replication and liver fibrosis of two groups of patients were analyzed before and after treatment, and the result showed that HBV-DNA loads as well as serum HA, LN, PC-III and IV-C levels of both groups of patients after treatment were lower than those before treatment and the HBV-DNA loads were not significantly different between the two groups, and serum HA, LN, PC-III and IV-C levels of combined treatment group were lower than those of western medicine treatment group. The results are consistent with the domestic scholar's research, and can indicate that Dahuang Zhechong pill treatment of chronic hepatitis b cirrhosis can slow the liver fibrosis process. But the drug has no significant effect on the hepatitis b virus replication, so it was speculated that Dahuang Zhechong pill might regulate immune response and inflammatory response to influence the process of liver fibrosis.

In the process of hepatitis b virus infection, the host antiviral immune response regulation disorder is the important pathological factor causing abnormal clearance and persistent infection of virus as well as liver fibrosis[8]. NK cell-mediated nonspecific immune response and T lymphocyte-mediated specific cellular immune response are the important mechanisms of the body to clear the virus. The activated NK cells can secrete perforin and cytotoxic factors to kill the virus and inhibit viral replication, and the reduced number and deficient activation of NK cells can affect hepatitis B virus clearance[9,10]. Treg cell is the T lymphocyte subset with immunosuppressive activities, which on the one hand, can suppress the differentiation and activation of multiple T lymphocyte subsets through direct contact inhibition, and be unfavorable to the activation of antiviral immune response, and on the other hand, secrete a variety of inhibitory cytokines to influence the killing effect of antiviral immune responses on the virus[11,12]. Studies have shown
that in the process of hepatitis b cirrhosis, the content of NK cells significantly decreases, and the content of Treg cells significantly increases[13]. In the above study, analysis of the immune cell levels in the peripheral blood between two groups of patients before and after treatment proved that peripheral blood NK cell levels of both groups after treatment were higher than those before treatment while Treg cell levels were lower than those before treatment, and peripheral blood NK cell levels of combined treatment group were higher than those of western medicine treatment group while Treg cell levels were lower than those of western medicine treatment group. This means that Dahuang Zhechong pill treatment of chronic hepatitis b cirrhosis can enhance the host antiviral immune response.

Abnormal host antiviral immune response would not only affect the virus clearance, but will also make mononuclear macrophages, hepatic stellate cells and kupffer cells abnormally secrete a variety of cytokines, cause hepatic extracellular matrix synthesis and degradation disorder, and accelerate the process of liver fibrosis. The IL-15 and IL-16 in local liver mainly come from the mononuclear macrophages, have high biological activity, and can activate the inflammation cascade activation in local tissue so as to induce the extracellular matrix remodeling and promote the development of liver fibrosis[14,15]; TGF-β, mainly comes from the hepatic stellate cells and kupffer cells, and can induce MMP2 and MMP9 activation and cause the synthesis and degradation disorder of a variety of proteins in extracellular matrix, which cause hepatic intercellular substance remodeling and fibrosis, and accelerate the process of liver cirrhosis[16,17]. Studies have shown that abnormal secretion of IL-15, IL-16 and TGF-β1 is closely related to the development of hepatitis b and the process of liver cirrhosis[18,19]. In the above study, analysis of serum levels of these cytokines between two groups of patients before and after treatment confirmed that serum IL-15, IL-16 and TGF-β1 levels of both groups after treatment were lower than those before treatment, and serum IL-15, IL-16 and TGF-β1 levels of combined treatment group were lower than those of control group. This means that Dahuang Zhechong pill treatment of chronic hepatitis b cirrhosis can adjust the secretion of inflammation-related cytokines so as to inhibit the process of liver fibrosis caused by inflammation disorder.

Dahuang zhechong pill combined with antiviral treatment of chronic hepatitis b cirrhosis can obtain the same antiviral effect as antiviral treatment.

References