Effect of nucleoside analogues in the treatment of hepatitis B cirrhosis and its effect on Th17 cells

J. ZHANG¹, H. YING¹, L. WEI¹, L.-J. HONG¹

Department of Emergency, The Capital Medical University Beijing YouAn Hospital, Beijing, China

Abstract. – OBJECTIVE: We conducted this study to analyze the effects of nucleoside analogues in the treatment of hepatitis B cirrhosis and its effect on Th17 cells.

PATIENTS AND METHODS: 120 patients were randomly divided into lamivudine combined with adefovir dipivoxil group (combined group) and entecavir group. There were 59 cases in the combined group and 59 cases in entecavir group. The combined group was administered lamivudine 100 mg/d + adefovir dipivoxil 10 mg/d and entecavir group was administered entecavir 0.5 mg/d. The treatment was continued until there was virus negativity and it maintains for at least 3 months.

RESULTS: The treatment effects were compared. We compared the average rate of viral clearance time and virus clearance of two groups of patients; the difference was not statistically significant (p>0.05). The relapse rate after a negative test of entecavir group was lower than that of the combined group (p<0.05). Before and after treatment, the levels of TBIL, ALT and ALB in the two groups were compared; the differences were not statistically significant (p>0.05). The Th17 cell proportion and the level of IL-17 after treatment of the entecavir group were lower than those before treatment. The combined group exhibited no change, and the entecavir group was lower than combined group; the differences were statistically significant (p<0.05).

CONCLUSIONS: Therefore, the effects of the combination of lamivudine and adefovir dipivoxil is the same as single entecavir treatment of hepatitis B cirrhosis suppression of viral replication. It does not increase liver injury and the antiviral effects of entecavir may be related to inhibition of the expression of Th17 cells and effector molecules IL-17.

Key Words: Hepatitis B cirrhosis, Lamivudine, Adefovir dipivoxil, Entecavir, Th17 cells, IL-17.

Introduction

Hepatitis B cirrhosis is the most common type of cirrhosis of the liver in China. The persistence of hepatitis B virus replication is the initiating and promoting factor that leads to the occurrence and aggravation of cirrhosis and the formation of hepatic carcinoma. Therefore, antiviral therapy is the key to hepatitis B cirrhosis. Nucleoside analogues are the preferred antiviral drugs in clinical application including lamivudine, adefovir, and entecavir. There are great differences in the antiviral effects and drug-resistance.

Due to the fact that lamivudine has high drug resistance, drug resistant patients taking the potent antiviral drug entecavir or adding adefovir dipivoxil for adjunctive therapy has been a major focus of clinical debate. The aim of this study was to analyze the therapeutic effects of two therapeutic schemes in the treatment of hepatitis B cirrhosis as well as to study the effect on immune function Th17 cells.

Patients and Methods

We continuously selected 120 hepatitis B cirrhosis patients that were diagnosed and treated for the first time in our hospital from January 2013 to January 2016. Inclusion criteria: (1) Consistent with the diagnostic criteria for the prevention and treatment of viral hepatitis; (2) Diagnosis of liver cirrhosis by imaging and tissue assessment, and the liver function was in compensatory stage; (3) Age between 18-75 years old; (4) The virus is active during the period of active replication, and HBeAg is positive.

Exclusion criteria: (1) Presence of other types of liver cirrhosis, such as alcoholic cirrhosis, autoimmune liver disease and fatty liver; (2) Presence of autoimmune disease such as immunodeficiency disease, combined with a variety of hepatitis viruses, genetic metabolic diseases, etc.; (3) Presence of serious heart, kidney, brain, lung and other organs dysfunction; (4) Inability
to tolerate the antiviral drug lamivudine, adefovir, entecavir. Shedding standard: (1) Failure to complete treatment; (2) Treatment needs to be replaced or terminated for exacerbations; (3) Clinical data is not perfect.

Subjects were randomly divided into the lamivudine combined with adefovir dipivoxil group (combined group) and entecavir group. There were 60 cases in each group with 2 cases of shedding in the combined group and 1 case of shedding in the entecavir group. Eventually, there were 58 cases included in the combined group and 59 cases in the entecavir group. In the combined group, there were 31 males and 27 females, aged 37-71 years old and on average (59.8 ± 12.6) years old. The viral load, on average, was 5-326 * 10^4 copy number/ml with an average of 54.7 ± 16.9 * 10^4 copy number/ml. In entecavir group, there were 30 males and 29 females. They were aged 38-72 years old with an average of 58.9 ± 14.6 years old. Their viral load was 8-296×10^4 copy number/ml, which averaged at 52.7 ± 18.5 210^4 copy number/ml. Baseline data in the two groups were comparable.

**Therapeutic Method**

Two groups of patients, according to their conditions, were treated for liver and gallbladder with drugs such as hepatocyte growth factor, potassium magnesium aspartate and the infusion of Human Albumin for both symptomatic and supportive treatment. Entecavir group’s scheme was Entecavir (ETV, baraclude, 0.5 mg, Bristol-Myers Squibb, 0.5 mg). 0.5 mg was administered orally, 1 time/day. We administered treatment continuously until there was virus clearance, which was maintained for at least 3 months.

**Observation Index**

**Virus Clearance time, Virus Clearance Rate, and Relapse Rate After Negative**

We recorded the average viral clearance time, the virus clearance rate of 3 months of treatment, and the relapse rate after a negative result of both groups of patients. HBV-DNA negative conversion standard is HBV-DNA value <1×10^3 copy number/ml.

**Liver Function**

After 3 months of treatment, the serum levels of total bilirubin (TBIL), alanine aminotransferase (ALT) and albumin (ALB) were detected. The routine biochemical analyzer (Beijing Liuyi Instrument Factory) was used for the determination.

**Th17 Cell Ratio and IL-17 Level**

After 3 months of treatment, we collected 3 ml of fasting peripheral venous blood. We separated 200 μl mononuclear cells, placed it in RPMI 1640 culture medium (Invitrogen Company, Carlsbad, CA, USA), added the working fluid (wave alcohol acetate, ionomycin, and monensin mycin) for mixing, and incubates it at 37°C, 5% CO₂, incubator (Bio-Rad Company, Hercules, CA, USA) for 4-6h. After removal, the PBS buffer was used to clean, and centrifuged at 3000 g for 20 min; precipitation cell was collected. We added 5 μl of CD4-FITC (fluorescein isothiocyanate labeled), 5 μl of IL-17-PE (phycoerythrin labeled), according to operation requirements of kit (Sigma-Aldrich Co., St. Louis, Mo, USA), and used flow cytometry (Beckman Coulter, Brea, CA, USA) to detect the proportion of Th17 cells. After taking supernatant, we detected interleukin-17 (IL-17) levels through the method of ELISA. The kit was obtained from Sigma-Aldrich Co., St. Louis, MO, USA.

**Statistical Analysis**

We used the SPSS20.0 software (SPSS Inc., Chicago, IL, USA) for statistics analysis. The measurement data was expressed by mean ± standard deviation and comparison between groups was tested by independent samples t-test. Comparison inside group used the paired t-test and the data counted was expressed by cases or (%) comparison between groups, which was tested by (correction) χ². p<0.05 indicated that the difference had statistical significance.

**Results**

**The Comparison of Viral Clearance Time, Viral Clearance Rate, and Relapse Rate After a Negative Result**

We compared the average viral clearance time and viral clearance rate of both groups of patients; the difference was not statistically significant (p>0.05). The relapse rate after a negative result of the entecavir group was lower than that of the combined group; the differences were statistically significant (p<0.05) (Table I).
Comparison of Liver Function Indexes

Before and after treatment, the levels of TBIL, ALT and ALB in the two groups were compared; the differences were not statistically significant ($p>0.05$) (Table II).

Comparison of Th17 Cell Proportion and IL-17 Levels

The Th17 cell proportion and the levels of IL-17 after treatment of the entecavir group were lower than those before treatment. Levels in the combined group were not significantly different, but the entecavir group had lower levels than the combined group; the differences were statistically significant ($p<0.05$) (Table III).

Discussion

About 25-40% of chronic hepatitis B patients progress to cirrhosis, which causes the liver function to be damaged. There may be other complications that emerge such as low protein blood, coagulation dysfunction, hormone inactivation abnormalities, esophageal gastric varices and others. Approximately 30-35% patients are associated with the occurrence of HCC. Guidelines for the treatment of hepatitis B cirrhosis emphasize that both compensatory and compensatory cirrhosis are required to be treated with antiviral therapy for a long period of time. Nucleoside analogues are the first choice as they are powerful and stable antiviral drugs. Lamivudine, adefovir dipivoxil, and entecavir have different specific pharmacological constituents; their antiviral mechanisms are also different.

Lamivudine has been known to play a role in inhibiting the HBV RNA-dependent polymerase; this study confirms that Lamivudine can have a positive antiviral effect on HBeAg negative or positive patients. However, some scholars found that lamivudine was ineffective in the clearance...

Table I. The comparison of viral clearance time, viral clearance rate, and relapse rate after negative.

<table>
<thead>
<tr>
<th>Group</th>
<th>The number of cases (n)</th>
<th>Virus clearance time (week)</th>
<th>Virus clearance rate [cases (%)]</th>
<th>Relapse rate after negative [cases (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entecavir group</td>
<td>59</td>
<td>28.6±3.4</td>
<td>57 (96.6)</td>
<td>4 (6.8)</td>
</tr>
<tr>
<td>Combined group</td>
<td>58</td>
<td>27.8±3.2</td>
<td>55 (94.8)</td>
<td>11 (19.0)</td>
</tr>
<tr>
<td>t/x²</td>
<td>0.271</td>
<td>0.000</td>
<td>3.886</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.615</td>
<td>0.984</td>
<td>0.049</td>
<td></td>
</tr>
</tbody>
</table>

Table II. Comparison of liver function indexes.

<table>
<thead>
<tr>
<th>Group</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entecavir group</td>
<td>25.6±4.3</td>
<td>24.3±4.5</td>
<td>35.6±3.7</td>
<td>34.7±3.9</td>
<td>46.8±5.3</td>
<td>48.9±5.2</td>
</tr>
<tr>
<td>Combined group</td>
<td>24.7±4.6</td>
<td>24.4±4.3</td>
<td>34.7±3.8</td>
<td>34.1±4.0</td>
<td>47.2±5.5</td>
<td>48.7±5.7</td>
</tr>
<tr>
<td>$t$</td>
<td>0.126</td>
<td>0.171</td>
<td>0.236</td>
<td>0.298</td>
<td>0.264</td>
<td>0.271</td>
</tr>
<tr>
<td>$p$</td>
<td>0.932</td>
<td>0.862</td>
<td>0.764</td>
<td>0.659</td>
<td>0.764</td>
<td>0.725</td>
</tr>
</tbody>
</table>

Note: TBIL, total bilirubin; ALT, alanine aminotransferase; ALB, albumin.

Table III. Comparison of Th17 cell proportion and IL-17 level

<table>
<thead>
<tr>
<th>Group</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entecavir group</td>
<td>4.5±0.7</td>
<td>3.4±0.6</td>
<td>256.7±35.7</td>
<td>150.6±28.5</td>
</tr>
<tr>
<td>Combined group</td>
<td>4.4±0.6</td>
<td>4.2±0.5</td>
<td>243.6±32.5</td>
<td>223.7±24.6</td>
</tr>
<tr>
<td>$t$</td>
<td>0.326</td>
<td>5.084</td>
<td>0.426</td>
<td>0.532</td>
</tr>
<tr>
<td>$p$</td>
<td>0.659</td>
<td>0.025</td>
<td>0.018</td>
<td></td>
</tr>
</tbody>
</table>
of the super spiral covalent, closed, circular DNA molecules (cccDNA) inside and outside liver cells; that may be due to the fact that the duration of treatment was short and the patients stopped the serum DNA HBV when they quickly recovered but before the end of the treatment. At the same time, about 20% of patients have developed resistance to lamivudine, which has become the biggest factor that restricts its clinical popularization\(^{11}\). Therefore, some authors support the change in regimen of potent the anti-viral drugs entecavir and more scholars believe that after the initial combination or resistance, adding adefovir dipivoxil can also get a better effect.

The study results show that the viral clearance time and the viral clearance rates of entecavir group and the combination group were not significantly different, suggesting that two drugs can have compatible and equal antiviral effects. Adefovir dipivoxil is commonly used compatible drugs of lamivudine; the mechanism of action is the inhibition of HBV DNA replication. In a global multi-center study\(^{12}\), patients with lamivudine resistance were treated with adefovir rescue therapy; results indicated that about 81% of the patients had no presence of HBV DNA and 76-83% of patients had their liver function return to normal. Before and after treatment, the levels of TBIL, ALT, and ALB in both groups were compared, and the differences were not statistically significant. Therefore, both therapeutic schemes are suggested to have a good protective effect on liver function. However, the latest research shows that\(^{13}\) adefovir dipivoxil has potential renal toxicity and during clinical treatment < 10 mg of dosage is often used to reduce the occurrence of renal dysfunction. However, the anti-viral speed of a small dose of adefovir dipivoxil is relatively slow, and so the ideal treatment effect requires a longer treatment time. Thus, this is a potential drawback of adefovir dipivoxil monotherapy\(^{14}\).

The relapse rate after negative is lower in the entecavir group, and the host immune response is considered to be related. The hepatitis virus is closely related to liver damage and the ability of the virus to replicate leads to toxic effects and the decreased ability of the host immune clearance\(^{15}\). Host immune response is the precursor to antiviral drugs as the cellular immune hepatic infiltration can not only directly induce liver injury but also promote inflammation induced by apoptosis of hepatic stellate cell activation, which speeds up or slows down the process of liver fibrosis\(^ {16}\). Some researches have confirmed that\(^ {17,18}\) in liver tissue of patients with liver cirrhosis, there are a large number of Th17 cells, which are CD4 helper T cell subsets and produced through the secretion of interleukin-17 (IL-17). They recruit inflammatory cells to the liver tissue, directly activate liver cells of the innate immune system, cause chronic inflammation of liver tissue and sustained damage of liver function. IL-17 has an important role in specific immunity towards infection of pathogens; it recruits monocytes and neutrophils and results in the induced generation of interleukin-6 (IL-6) and promotes T cell infiltration\(^ {19}\). The increase in the number of peripheral blood Th17 cells may be an important mechanism of cirrhosis and virus re-positive as the content of IL-17 as an effector molecule of Th17 cells in peripheral blood significantly increases. Elevated Th17 cells and IL-17 levels are positively correlated with viral load in patients with cirrhosis\(^ {20}\). The study concluded that after treatment of the entecavir group, the Th17 cell proportion and the levels of IL-17 were reduced when compared to before treatment, the combined group had no change, and the entecavir group was lower than the combined group.

**Conclusions**

The effect of the combination of lamivudine and adefovir dipivoxil is the same as entecavir monotherapy of hepatitis B cirrhosis through the suppression of viral replication and no liver injury. The antiviral effects of entecavir may be related to inhibition of the expression of Th17 cells and the effector molecule IL-17. Further analysis of the role of the two anti-virus programs in the decompensation stage of hepatitis B cirrhosis is needed.

**Conflict of interest**

The authors declare no conflicts of interest.

**References**


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