Effect of adefovir dipivoxil on T cell immune function in the treatment of chronic hepatitis B and hepatocirrhosis

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Abstract. The aim of the present study was to investigate the T cell immune function in chronic hepatitis B hepatocirrhosis patients at the compensated and decompensated stage following treatment with adefovir dipivoxil. A total of 104 patients diagnosed with hepatitis B hepatocirrhosis during the period from October 2013 to October 2014 were enrolled in the study. Among the cases, there were 56 cases at compensated stage, and another 48 at decompensated stage. Adefovir dipivoxil was administered for antiviral therapy (10 mg/time, 1 time/day, for a total of 24 weeks), and we compared the virus disappearance rate, liver function improvement and T cell immune function between the two groups before and after treatment. The difference between the virus disappearance rate in the two groups was not statistically significant (P>0.05). The decreased level of ALT decrease in the compensated group was significantly higher than that in the decompensated group, while the increased level of albumin in the compensated group was significantly higher as well. The differences showed statistical significance (P<0.05). After treatment, the level of CD4$^+$ and CD4$^+$/CD8$^+$ ratio were higher than before treatment, while the level of CD8$^+$ was lower after treatment than before treatment in the two groups. The differences all showed statistical significance (P<0.05). The CD4$^+$CXCR5$^+$ T follicular helper (TFH) cell level in the two groups was higher after treatment, as was interleukin-2 and interferon-γ. The differences all showed statistical significance (P<0.05). As for comparison between groups, the difference had no statistical significance (P>0.05). Adefovir dipivoxil treatment can improve T cell immune function at the compensated and decompensated stages in chronic hepatitis B hepatocirrhosis patients. This may be associated with virus disappearance and liver function improvement.

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Introduction

The nucleotide analog adefovir dipivoxil has a clear-cut effect in treating acute and chronic hepatitis B hepatocirrhosis and has shown many advantages, such as clinical strengths of low drug resistance and light side effects (1,2). Apart from interfering with the DNA transcription directly, adefovir dipivoxil can adjust the immune function of the body, including cellular and humoral immunity (3). A previous study indicated that adefovir dipivoxil can improve the level of CD4$^+$ and CD4$^+$/CD8$^+$ and lower the level of CD8$^+$, thus playing an important role in antivirus activity and reversal of hepatic fibrosis (4). Based on this, we hypothesized that adefovir dipivoxil may be able to adjust and interfere with the T cell immune function in chronic hepatitis B hepatocirrhosis patients. The development and progression of hepatitis B virus (HBV)-related hepatic cirrhosis involves not only damage to liver cells and the body's immune system induced by viral replication but also virus cleaning and self-repairing by the body (5). The extent of liver function lesions is not consistent with the process of viral replication (6). The immune system, especially T cells, plays a vital role in the process of viral replication. To date, few studies have investigated the immune capacity of T cells at different states of liver function and whether the metabolism and activation capacity of different antiviral drugs are consistent (7,8).

The purpose of the present study was to investigate the effects of adefovir dipivoxil on patients with HBV-related hepatocirrhosis at different stages and to explore the regulating capacity of adefovir dipivoxil on immune function of T lymphocytes.

Patients and methods

Patients. A total of 104 patients diagnosed with hepatitis B hepatocirrhosis during the period from October 2013 to October 2014 were enrolled in the study. Inclusion and exclusion criteria were as follows. Inclusion standards include: i) age, ≥18 and ≤75 years; ii) fulfills the diagnostic criteria of liver cirrhosis and stage is known; and iii) receives antivirus treatment for the first time. Exclusion standards include: i) patients with non-hepatitis B hepatocirrhosis, alcoholic hepatitis B, autoimmune liver disease, development into cancer; ii) patients presenting complications of serious functional

Table I. Virus quantity and comparison of the disappearance rate.

Groups	HBV DNA level (10^4 copies/ml)				HBsAg, HBeAg, HBcAb-positive (cases, %)			
	Before treatment	After treatment	t	P-value	Before treatment	After treatment	χ^2	P-value
Compensated (n=56)	23.4±5.2	2.2±0.3	68.624	<0.001	49 (87.5)	11 (19.6)	51.836	<0.001
Decompensated (n=48)	23.6±6.3	2.5±0.4	66.462	<0.001	45 (93.8)	12 (25.0)	47.028	<0.001
t (χ^2)	0.547	0.109			0.554	0.431		
P-value	0.326	0.748			0.457	0.512		

HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; HBcAb, hepatitis B core antibody.

Table II. Comparison of liver function improvement.

Groups	ALT (U/l)					Albumin (g/l)				
	Before treatment	After treatment	t	P-value	Improvement	Before treatment	After treatment	t	P-value	Improvement
Compensated (n=56)	46.5±4.7	36.5±3.3	3.648	0.041	11.6±2.7	43.7±6.2	60.2±5.2	4.827	0.038	18.2±4.4
Decompensated (n=48)	77.8±6.9	69.2±5.4	3.325	0.042	7.5±3.3	22.5±3.4	35.4±3.9	4.629	0.039	11.6±3.2
t	4.321	4.627			5.624	5.026	5.424			5.794
P-value	0.039	0.036			0.027	0.034	0.032			0.025

ALT, alanine aminotransferase.

disorder of the heart, liver, kidney or other organs; adefovir dipivoxil cannot be tolerated and have no drug resistance; and iii) patients who do not abide by the study regulations or provide full information, or reject to participate in the study.

Hepatitis B hepatocirrhosis was divided into the compensated stage and decompensated stage according to the disease stage. Fifty-six patients were in the compensated stage: 30 males and 26 females, aged from 37 to 66 years with an average age of 49.8±10.3 years. The course of disease in this group ranged from 1 month to 10 years, with an average of 3.2±0.6 years. Forty-eight patients were in decompensated stage: 25 males and 23 females, aged from 36 to 74 years with an average age of 50.2±12.2 years. The course of disease in this group ranged from 6 months to 13 years, with an average of 3.9±1.2 years. No statistical significance was found in terms of gender, age and disease course between these two groups (P>0.05).

Methods. Proper treatment was administered to the patients. For example, anemia patients with <60g/l hemoglobin were given a blood transfusion. Patients with low protein received an external protein supplement. The patients received a healthy diet and were well-rested with protection of the liver with lowering of transaminase level. Adefovir dipivoxil (10 mg/time, 1 time/day, for a total of 24 weeks; Zhengda Tianqing Pharmaceutical

Co., Ltd., Nanjing, China) was administered as antivirus treatment. A routine blood examination, liver and kidney function as well as the virual replication level were regularly supervised.

Observation index and test method. Differences in the virus disappearance rate, liver function improvement and T cell immune function were analyzed. Virus disappearance occurs when positive HBsAg, HBeAg, HBcAb levels become negative. By testing the ALT level before and after treatment and the level of albumin, the condition of the liver function can be assessed. The ELISA method was applied for HBV serum marker. The kit was provided by Yingke Xinchuang Technology Co., Ltd. (Xiamen, China). Olympus full-automatic analyzer AU5400 SOP was applied as a standard in testing the liver function. The HBV DNA quantitative test uses the fluorescent quantitative polymerase chain reaction method. The kit was provided by DaAn Gene Co., Ltd. of Sun Yat-Sen University (Guangzhou, China). The lowest limit of detection is 10^3 copies/ml. The T cell immune function was reflected by the level of CD4^{+}, CD8^{+}, CD4^{+}/CD8^{+} and CD4^{+}CXCR5^{+} T follicular helper (TFH) cells, interleukin (IL)-2 and interferon (IFN)-γ. The test for the subpopulation of T cells was carried out using EPICS XL flow cytometry analyzer and System II software (both from Beckman Coulter, Inc., Brea, CA, USA).

Table III. Comparison of CD4^{+} and CD8^{+} levels and CD4^{+}/CD8^{+} ratio.

Groups	CD4^{+}				CD8^{+}				CD4^{+}/CD8^{+}			
	Before treatment	After treatment	t	P-value	Before treatment	After treatment	t	P-value	Before treatment	After treatment	t	P-value
Compensated (n=56)	32.4±3.2	41.5±4.3	5.123	0.027	26.8±3.6	21.4±2.5	4.714	0.033	1.3±0.3	2.4±0.5	6.632	0.024
Decompensated (n=48)	30.5±3.1	39.8±3.8	5.624	0.023	27.7±3.4	19.9±2.4	4.623	0.035	1.1±0.2	2.2±0.4	6.325	0.026
t	0.329	0.621			0.748	0.936			0.203	0.417		
P-value	0.528	0.749			0.419	0.537			0.636	0.958		

Table IV. Comparison of CD4^{+}CXCR5^{+} TFH cell, IL-2 and IFN-γ levels.

Groups	CD4^{+}CXCR5^{+} TFH cells (%)				IL-2 (ng/ml)				IFN-γ (pg/ml)			
	Before treatment	After treatment	t	P-value	Before treatment	After treatment	t	P-value	Before treatment	After treatment	t	P-value
Compensated (n=56)	21.6±4.2	29.7±3.6	4.785	0.039	45.7±6.9	89.6±10.2	5.928	0.027	67.8±14.5	102.4±22.5	5.624	0.033
Decompensated (n=48)	18.8±3.7	26.5±3.2	4.628	0.041	43.2±6.3	85.5±11.3	5.627	0.029	63.4±15.3	93.7±20.3	5.327	0.037
t	0.625	0.364			0.747	0.858			0.758	0.629		
P-value	0.424	0.527			0.624	0.235			0.231	0.412		

IL, interleukin; IFN, interferon; TFH, follicular helper.

Statistical analysis. SPSS statistics 19.0 was applied to analyze the data. The measurement data were expressed as the mean ± standard deviation. The t-test was carried out to analyze differences between groups while the unit was number of cases or percentage. The χ^2 test was applied for inter-group comparison. P<0.05 was considered to indicate a statistically significanct difference.

Results

Virus quantity and comparison of the viral disappearance rate. The difference in the HBV DNA quantitative level of the the compensated and decompensated stage groups before and after treatment of adefovir dipivoxil, as well as the positive rate of HBsAg, HBeAg and HBcAb did not show statistical significance (P<0.05). After treatment, the HBV DNA quantitative level and the positive rate of HBsAg, HBeAg and HBcAb were obviously decreased, showing statistical significant differences (P<0.05). Yet, there were no statistically significant differences in inter-group comparison (Table I).

Comparison of liver function improvement. The altered level of ALT and albumin before and after treatment represented liver function improvement. The liver function in both groups was improved after treatment. The ALT improvement in the compensated group was obviously higher than that noted in the decompensated group, while the increase in albumin in the compensated group was significantly obvious as well. The differences showed statistical significance (P<0.05) (Table II).

Comparison of T cell immune function. Before adefovir dipivoxil treatment, the difference in T cell immune function between the two groups showed no statistical significance. After treatment, the level of CD4^{+} and CD4^{+}/CD8^{+} were higher than before treatment, while the level of CD8^{+} became lower. The differences showed statistical significance (P<0.05). The CD4^{+}CXCR5^{+} TFH cell levels in the two groups were higher after treatment, as were IL-2 and IFN-γ. The differences showed statistical significance (P<0.05). As for comparison between groups, the difference was not statistically significant (P>0.05) (Tables III and IV).

Discussion

The pathogenesis of chronic hepatitis B combined with hepatic cirrhosis is complex. In addition to the pathogenic effects of the virus, immune function disturbance also plays an important role. The immune response not only removes viruses, but also causes immune injury to hepatic cells, and may even cause virus mutation (9). The balance system of T lymphocyte subsets plays an important role in the removal of HBV (10).

CD4^{+} T lymphocytes identify exogenous antigenic peptides presented by MHC-II molecules, and then mainly differentiate into Th cells after activation (11). CD8^{+} T lymphocytes identify

endogenous antigenic peptides presented by MHC-I molecules, and then mainly differentiate into CTL cells after activation (11). After the body is infected by HBV, the virus replicates in host cells and produces viral proteins (12). MHC-I molecules in cells combine with these proteins selectively to form complexes. These complexes are identified by specific antigens of T lymphocytes, which then activate CD8^{+} T lymphocytes which cause injury to hepatic cells (13). At the same time, CD4^{+} T lymphocytes mediate activation of B lymphocyte by MHC-II molecules on their surfaces, and promote B lymphocytes to produce anti-HBs which helps to clear HBV (14). This is the major reason why HBV causes injury to hepatic cells, and this is also the major mechanism involved in the host removal of the virus in the cells. Cell levels of CD4^{+} and CD8^{+} of T lymphocyte subsets in peripheral blood and the ratio of CD4^{+}/CD8^{+} reflect the cellular immunity state of the body. Changes in T lymphocyte subsets in peripheral blood of patients with chronic hepatitis B reflect immunopathological changes in the patient cellular immunity (15).

Moreover, recent research has reported that T follicular helper (TFH) cells are the main T-cell subset that instruct B helper cells to produce antibody (16). These cells are located in lymph follicles and colocalized and interacted with B cells. They help B cells to participate in humoral immune response by continuously expressing membrane surface molecules such as CXCR5/ICOS/PD-1 and producing cytokines including IL-21. Adefovir dipivoxil is a purine derivative. It is hydrolyzed to dissociated adefovir by esterase quickly after oral intake. It then enters portal veins and the systemic circulation. It can be bisphosphonated to an active metabolite which is adefovir dipivoxil bisphosphonate by cellular kinases. Adefovir dipivoxil bisphosphonate competes with the natural substrate deoxyadenosine triphosphate (dATP) to inhibit the activity of HBV DNA polymerase and integrates it into the viral DNA strand to stop it from synthesis and inhibition of virus replication (17). In addition, adefovir is able to induce endogenous INF, increase natural killer cell activity and stimulate the body's immune response. Therefore, it has strong anti-HBV function and it is a safe and effective medicine for decompensated liver cirrhosis patients (18).

The present study is innovative as it explores the immune function of T lymphocytes when B liver cirrhosis patients in the compensated and decompensated period are treated with adefovir dipivoxil. The results showed that differences in the virus negative conversion ratio in the two groups did not achieve statistical significance; for the compensated group, the decreased level of ALT and increased level of albumin were obviously higher than those in the decompensated period and the differences were statistically significant. After treatment, the level of CD4^{+} and the CD4^{+}/CD8^{+} ratio in the two groups were higher than before treatment while the level of CD8^{+} was lower, however, no statistical significance was noted regarding differences between the two groups. CD4^{+}CXCR5^{+} TFH cell, IL-2 and IFN-γ levels were higher than before treatment but differences were not statistically significant between groups. In conclusion, adefovir dipivoxil can improve the immune function of T lymphocytes in chronic hepatitis B hepatocirrhosis patients in the compensated and decompensated periods, which may be related to negative conversion of the virus and improvement in liver function.

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