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Original Research Article

Efficacy of tenofovir dipivoxil plus entecavir in patients with HBeAg positive chronic hepatitis B

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Abstract

Purpose: *To investigate the efficacy of tenofovir disoproxil fumarate (TDF) plus entecavir in patients with HBeAg positive chronic hepatitis B.*
Methods: *A total of 124 patients with HBeAg-positive chronic hepatitis who were hospitalized at The First People's Hospital of Jiangxia District, Wuhan, China were chosen as the subjects. They were then randomized equally into study and control groups. Control group received entecavir (0.5 mg orally) while the study group administered TDF (300 mg orally) daily for 48 weeks. Efficacy, liver function, inflammatory factors, liver fibrosis, and adverse reactions between the two groups were evaluated.*
Results: *After 12 weeks of treatment, HBeAg and HBV DNA negative seroconversion rates were significantly higher in the study group compared to control group ($p < 0.05$). At 24 and 48 weeks after treatment, alanine transaminase (ALT) normalization rate, HBeAg and HBV DNA negative seroconversion rate were also significantly higher compared to control group. Levels of heat shock protein 47 (HSP47), endothelial nitric oxide synthase (eNOS), and major basic protein (MBP) were significantly lower in study group compared to control group. Levels of hyaluronan (HA), type IC collagen (IV-C), N-terminal propeptide of procollagen type III (PIIINP), and laminin (LN) were significantly lower in study group compared to control group ($p < 0.05$). There was no significant difference in incidence of adverse reactions between the two groups ($p > 0$ 05).*
Conclusion: *The combination of ETV and TDF significantly inhibits hepatitis B virus replication, and reduces inflammatory reactions. Further studies to determine the mechanism of action of tenofovir and entecavir on hepatitis B virus would be required.*

Keywords: *Tenofovir disoproxil fumarate, Entecavir, High viral load, HBeAg positive, Chronic hepatitis B*

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INTRODUCTION

The term "chronic hepatitis B" or "chronic viral hepatitis B" refers to a chronic illness in which the liver exhibits varying degrees of fibrosis or inflammation and a positive hepatitis B virus (HBV) test [1]. Some studies have reported that there are 120 million carriers of chronic hepatitis B virus in China, of which 30 million are CHB patients, with less than one-tenth undergoing treatment [2]. Presence of hepatitis B e antigen (HBeAg) indicates high levels of the virus in the blood. Viral load (VL) is the number of viruses

per milliliter of blood, and the VL level shows progression of the disease. Thus, a high viral load (HVL) means that the virus is still replicating in the blood [3].

Chronic hepatitis B (CHB) is one of the world's major infectious diseases, and it poses a serious health threat to human lives [4]. If HBeAg is persistently positive, it means that the liver cells are severely damaged, and the higher the viral load, the higher the likelihood of cirrhosis or even hepatocellular carcinoma. As a result, there is an urgent need to find a treatment program that is both effective and safe. In recent years, antiviral therapy such as entecavir (ETV) and Tenofovir disoproxil fumarate (TDF) is mostly given depending on the patient's condition [4].

Entecavir (ETV) suppresses viral replication, has a low incidence of drug resistance, and no evident adverse effects, while TDF inhibits viral polymerase and has a robust antiviral effect [5]. Thus, this study was aimed at investigating the effects of tenofovir disoproxil fumarate plus entecavir compared to using entecavir alone in the treatment of HBeAg-positive CHB patients.

METHODS

General information

One hundred and twenty-four cases of HBeAg-positive CHB patients admitted to First People's Hospital of Jiangxia District, Wuhan, China were selected for the study. They were randomly and equally divided into study and control groups. Study group consisted of 32 males and 30 females aged 18 - 65 years, with an average age of 48.32 ± 4.42 years. Control group consisted of 31 males and 31 females aged 18 - 65 years, with a mean age of 48.27 ± 4.30 years. In terms of gender, age, disease duration, and symptoms; differences between the two groups were not significant. The study protocol was approved by the Ethics Committee of The First People's Hospital of Jiangxia District, Wuhan City (Union Jiangnan Hospital Huazhong University of Science and Technology) (approval no. 2021101), and complied with the guidelines of Declaration of Helsinki [6]. Written informed consent was obtained from legally authorized representative(s) for anonymized patient information to be published in this article.

Inclusion criteria

Meeting the diagnostic criteria of chronic hepatitis B, disease duration of more than 1 year, not taking nucleoside analogs, and signed informed consent form to participate in the study.

Exclusion criteria

Other viral hepatitis, liver diseases, kidney diseases, and cognitive disorders.

Treatment

Control group received 0.5 mg orally dispersible entecavir tablets (Zhengda Tianqing Pharmaceutical Group, National Drug License no. H20100019) daily for 48 weeks. Study group received 300 mg orally administered tenofovir disoproxil fumarate (Beit Pharmaceuticals, approval no. H20163436) once a day for 48 weeks in addition to entecavir. In both groups, venous blood was collected during fasting, and the serum was separated after centrifugation. The data of the relevant indices of the two groups were then recorded and compared at the 4^{th}, 20^{th}, 24^{th}, and 48^{th} weeks respectively.

Evaluation of parameters/indices

Serum HBeAg level

Fluorescence quantification was used to assess HBV DNA, and an enzyme-linked immunosorbent assay was used to measure ALT. If serum HBeAg level was not detected, it indicated that the HBeAg was negative. If serum HBeAg level was not detected or was fewer than 100 copies/mL, it indicated that HBV DNA had been transmitted. The ALT recovery rates, HBeAg and HBV DNA seroconversion rates were then examined and compared.

Liver function

A full-wavelength enzyme marker was used to quantify hepatitis B surface antigen. A color doppler ultrasound diagnostic instrument was used to measure liver stiffness, and spleen thickness. The quantitative value of hepatitis B surface antigen, liver stiffness and spleen thickness of the two groups were compared.

Inflammatory factor levels

Radio-immunoassay was used to determine levels of heat shock protein 47 (HSP47), endothelial nitric oxide synthase (eNOS), and major basic protein (MBP) and the values were compared in both groups.

Liver fibrosis level

Radio-immunoassay was used to detect levels of hyaluronan (HA), type IC collagen (IV-C), N-terminal propeptide of procollagen type III

(PIIINP), and laminin (LN) in serum and the values compared in both groups.

Adverse reactions

The incidence of adverse reactions was compared between the two groups.

Statistical analysis

Study data were analyzed and processed by Statistical Packages for Social Sciences (SPSS version 22.0, IBM, Armonk, NY, USA). Measurement data were presented as mean ± standard deviation (SD) and anlysed using t-test. Count data were expressed as frequency and percentages and analysed using chi-square test. *P < 0.05* was considered statistically significant.

RESULTS

Efficacy

At the 24th and 48th week of treatment, the study group showed significantly greater rates of ALT recovery (Table 1), HBeAg seroconversion (Table 2), and HBV DNA seroconversion rate (Table 3) compared to the control group (p < 0.05).

Liver function

Before treatment, there was no significant difference in levels of hepatitis B surface antigen, liver stiffness, and spleen thickness between the two groups (p < 0.05). However, after treatment, study group had significantly lower levels of hepatitis B surface antigen, liver stiffness, and spleen thickness compared to control group (p < 0.05).

Levels of inflammatory factors

After treatment, levels of HSP47, eNOS, and MBP were significantly lower in study group compared to control group (p < 0. 05) (Table 5).

Levels of liver fibrosis markers

Levels of liver fibrosis markers (HA, IV-C, PIIINP, and LN) were significantly lower in study group compared to control group (p < 0.05) (Table 6).

Incidence of adverse reactions

There was no significant difference (p > 0.05) in incidence of adverse reactions between the two groups (Table 7).

Table 1: Alanine transaminase (ALT) recovery rate (n = 62 in each group)

Group	ALT reversion rate			
	4 weeks	***12 weeks***	***24 weeks***	***48 weeks***
Study	45(72.58)	46(74.19)	56(90.32)	60(96.77)
Control	36(58.06)	42(67.74)	43(69.35)	49(79.03)
χ^2	2.884	5.835	8.467	9.177
P-value	0.090	0.429	0.004	0.003

Table 2: The HBeAg seroconversion rate (N = 62 in each group)

Group	HBeAg seroconversion rate			
	4th week	***12th week***	***24th week***	***48th week***
Study	28(45.16)	40(64.52)	44(70.97);	49(79.03)
Control	24(38.71)	27(43.55)	30(46.77)	35(56.45)
χ^2	0.530	5.487	6.569	7.233
P-value	0.467	0.019	0.010	0.007

Table 3: Hepatitis B viral DNA seroconversion rate (N = 62 in each group)

Group	HBV DNA seroconversion rate			
	4th week	***12th week***	***24th week***	***48th week***
Study	45(72.58)	51(82.26)	59(95.16)	60(96.77)
Control	36(58.06)	38(61.29)	48(77.42)	51(82.25)
χ^2	2.884	6.727	8.249	6.961
P-value	0.090	0.010	0.004	0.008

Table 4: Liver function (N = 62 in each group)

Group	Hepatitis B surface antigen quantification (ng/mL)		Liver stiffness value (kPa)		Spleen thickness (cm)	
	Before Treatment	***After Treatment***	***Before Treatment***	***After Treatment***	***Before Treatment***	***After Treatment***
Study	12.28±2.85	3.86±1.02	42.46±6.25	10.85±2.96	22.15±4.23	14.15±2.86
Control	12.24±2.83	5.45±1.18	42.45±6.27	14.44±2.35	22.14±4.25	16.94±2.77
T-value	0.078	8.027	0.000	7.479	0.013	5.518
P- value	0.938	<0.001	1.000	<0.001	0.990	<0.001

Table 5: Levels of inflammatory factors (N = 62 in each group)

Group	HSP47		eNOS		MBP	
	Before treatment	***After treatment***	***Before treatment***	***After treatment***	***Before treatment***	***After treatment***
Study	82.13±9.35	30.36±5.16	112.78±15.25	62.81±7.84	29.18±5.13	9.15±2.06
Control	82.17±9.33	37.15±6.12	112.75±15.27	70.14±8.82	29.16±5.17	13.24±2.94
T-value	0.024	6.679	0.011	4.891	0.022	8.971
P-value	0.981	<0.001	0.991	<0.001	0.983	<0.001

Table 6: Levels of liver fibrosis markers (ng/mL) (N =62 in each group)

Groups	HA		IV-C		PⅢNP		LN	
	Before treatment	***After treatment***	***Before treatment***	***After treatment***	***Before treatment***	***After treatment***	***Before treatment***	***After treatment***
Study	216.64±15.85	119.36±15.26	273.28±20.92	131.75±21.57	64.38±6.13	26.15±5.06	335.21±26.76	185.62±25.74
Control	216.67±15.83	154.25±14.12	273.22±20.96	181.24±22.13	64.36±6.17	41.04±5.94	335.24±26.71	275.36±25.66
T-value	0.011	13.214	0.016	12.559	0.018	15.026	0.006	19.442
P-value	0.992	<0.001	0.987	<0.001	0.986	<0.001	0.995	<0.001

Table 7: Incidence of adverse reactions

Group	Creatine kinase elevation	Headache	Rash	Epigastric pain	Incidence of adverse effects
Study	3(4.83)	2(3.23)	3(4.83)	1(1.61)	9(14.52)
Control	5(8.06)	3(4.83)	4(6.45)	2(3.23)	14(22.58)
χ^2					1.335
P-value					0.248

DISCUSSION

Chronic viral Hepatitis B (CHB) is transmitted through blood transfusion, mother-to-child, and sexual contact. In recent years, with increase in hepatitis B vaccination, rate of new CHB patients has decreased significantly [7]. However, due to lack of promotion of hepatitis B vaccination in the 1990s, there is still a sizable pool of CHB patients in China, with some of the new patients contracting the disease through mother-to-child transmission [8]. In clinical practice, there are two main antiviral therapeutic drug classes for CHB. One of them is interferon, which has a good clearance rate for HBsAg and HBeAg but has low cure rate, requires injections, is more expensive, and has more adverse effects [9]. The other type are nucleoside analogs, which are administered orally to provide long-term viral suppression. Oral drugs are becoming increasingly popular in clinics due to their low cost, safety, and less adverse effects. The drawback of these oral drugs is that repeated drug use may lead to drug resistance or other negative side effects [10].

For CHB patients, the most important thing is to stop further development of the disease and reduce the risk of developing cirrhosis or hepatocellular carcinoma. Inflammation is controlled to an extent by suppressing viral replication and liver fibrosis. Entecavir (ETV) and Tenofovir Disoproxil Fumarate (TDF) are currently the first-line antiviral drugs for the clinical treatment of CHB [11]. Entecavir (ETV) is a guanine nucleoside analog that reacts with deoxyguanosine triphosphate to inhibit activity of viral polymerase (reverse transcriptase). Some studies have however shown that repeated ETV administration may lead to drug resistance [12]. Tenofovir (TDF) is a novel nucleotide reverse transcriptase inhibitor-containing tenofovir bisphosphate, which inhibits viral polymerase by directly and competitively binding to the natural deoxyribose substrate to prevent viral replication [13,14].

This study demonstrated that in patients with HBeAg-positive CHB, the combination of TDF and ETV was more effective compared to ETV alone. Differences in ALT recovery rate, HBeAg and HBV DNA seroconversion rate between the two groups were not significant at 4 and 12 weeks after treatment, but they became significant at 24 and 48 weeks after treatment as a result of the longer treatment period and sustained effects of the medication. The study group also showed significant higher ALT recovery rate. The HBeAg and HBV DNA seroconversion rate was significantly in study group compared to control group, indicating that the combination of TDF and ETV was more effective. Further investigation revealed that combination of TDF and ETV blocks the activity of viral polymerase, resulting in interruptions in DNA replication and, ultimately, inhibiting effective replication process of hepatitis B virus. This study demonstrates that combination of TDF and ETV improves liver function, liver stiffness value, and spleen thickness of the two groups after treatment. Differences in levels of HSP47, eNOS, and MBP of study and control groups were significant after treatment although lower in study group compared to control group. Long-term infection with hepatitis B virus is accompanied by a variety of inflammatory response processes [15]. Patients commonly have HSP47, a type of molecular chaperone with collagenous properties, in their endoplasmic reticulum and many organs. Furthermore, eNOS is a small molecule protein found commonly in endothelial tissue of blood arteries. Elevated HSP47 levels in patients indicate that the liver is damaged with increased risk of bleeding [16].

The function of MBP is to maintain a state of homeostasis in structural activities of the neurological system, hence, it might induce a greater generation of oxidized free radicals in the vascular system, resulting in increased damage to the hepatic vascular system. Also, MBP enters the bloodstream through the myelin sheath to repair an imbalance in the the structure and function of the nervous system when nerve function is abnormal. Levels of MBP are elevated in people with liver problems or patients with hepatic peripheral nerve system dysfunction [17]. The results of this study further indicate that combination of TDF and ETV reduces inflammatory factor levels in patients and effectively controls inflammatory process, which is consistent with previous findings [16].

This study also showed that there was a significant difference in HA, IV-C, PIIINP, and LN levels between the two groups after treatment; suggesting that the combination of TDF and ETV reduces levels of hepatic fibrosis, decreases liver damage, and reduces possibility of further disease progression. Clinical studies have found that HA, IV-C, PIIINP, and LN are all products that form at different stages of the liver fibrosis process [18]. Changes in levels of these products serve as key markers in clinical settings to indicate liver fibrosis. Combination of TDF and ETV reduces inflammatory response of the liver, decreases stimulation of intrahepatic stellate cells, reduces deposition of intrahepatic collagenous extracellular matrix, and improves the process of fibrosis which are consistent with earlier findings [19].

Limitations of the study

The limitations of this study are small sample size and short duration of the observation period; particularly in the examination of the mechanism of action of tenofovir and entecavir on the hepatitis B virus. This will be one of the areas that future study should investigate.

CONCLUSION

Combined therapy of ETV plus TDF effectively inhibits hepatitis B virus replication, reduces inflammatory response, and improves the process of hepatic fibrosis with higher safety profile. Further studies should determine the mechanism of action of tenofovir and entecavir on hepatitis B virus.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Xiang Deng, Hua Liu and Zaihui Jiang designed the study and carried them out, supervised the data collection, analyzed and interpreted the data, prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the final of the manuscript for publication. Xiang Deng and Hua Liu contributed equally to the work and should be considered co-first authors.

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