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Original Article

Diwu Yanggan capsule improving liver histological response for patients with HBeAg-negative chronic hepatitis B: a randomized controlled clinical trial

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Abstract: The number of patients with hepatitis B e antigen (HBeAg)-negative chronic hepatitis B (CHB) has shown a significant upward trend in recent years. However, antiviral drugs are not very effective. Regulation of liver regeneration by traditional Chinese medicine is an important way to improve clinical efficacy. This randomized controlled trial assessed the efficacy and safety of DWYG in patients with HBeAg-negative CHB. Overall, 130 subjects were randomized to (A) DWYG 1.2 g three times daily (n = 44), (B) entecavir 0.5 mg/day (n = 43) in combination with DWYG or (C) entecavir 0.5 mg/day (n = 43). The liver histological response rate was assessed as the primary efficacy endpoint. The results showed that the liver histological response rate in the combination treatment group was significantly higher than that in the group with entecavir (71.43% versus 22.22%; P = 0.036) after 48 weeks of treatment. And the pathological progression rate of liver in the group with DWYG was significantly lower than that of the entecavir group during 228 weeks of follow-up (0% versus 60.00%; P = 0.019). No significant adverse events occurred during the study. In conclusion, treating HBeAg-negative CHB with DWYG is safe and effective to improve liver histological response.

Keywords: Hepatitis B e antigen-negative chronic hepatitis B, clinical trial, Diwu Yanggan capsule, liver regeneration, liver histological biopsy

Introduction

Chronic hepatitis B virus (HBV) infection is a serious public health problem worldwide, but the prevalence of chronic HBV infection varies greatly by region. The World Health Organization recently reported that more than 2 billion people worldwide have been infected with HBV, of which 240 million people are infected with chronic HBV [1]. Each year, approximately 650,000 people die of liver failure, cirrhosis and liver cancer caused by HBV infection [2]. The proportions of patients with cirrhosis and liver cancer caused by HBV infection worldwide are 30% and 45%, respectively [2, 3]. However, the proportions in China are up to 60% and 80%, respectively [4]. In recent years, the acute HBV infection rate has been significantly reduced due to the hepatitis B vaccine. The age of the population infected with HBV and the widespread application of antiviral therapy have led to a rising proportion of patients with hepatitis B e antigen (HBeAg)-negative chronic hepatitis B (CHB) [5]. Currently, the number of patients with HBeAg-negative CHB is more than half of all patients with CHB and shows a rapid increasing trend.

In the past, HBeAg seroconversion was considered to indicate a reduction in viral replication, and hepatic inflammation tended to decrease and resolve. Recent studies have found many differences between HBeAg-positive CHB and HBeAg-negative CHB in the etiology, epidemiol-

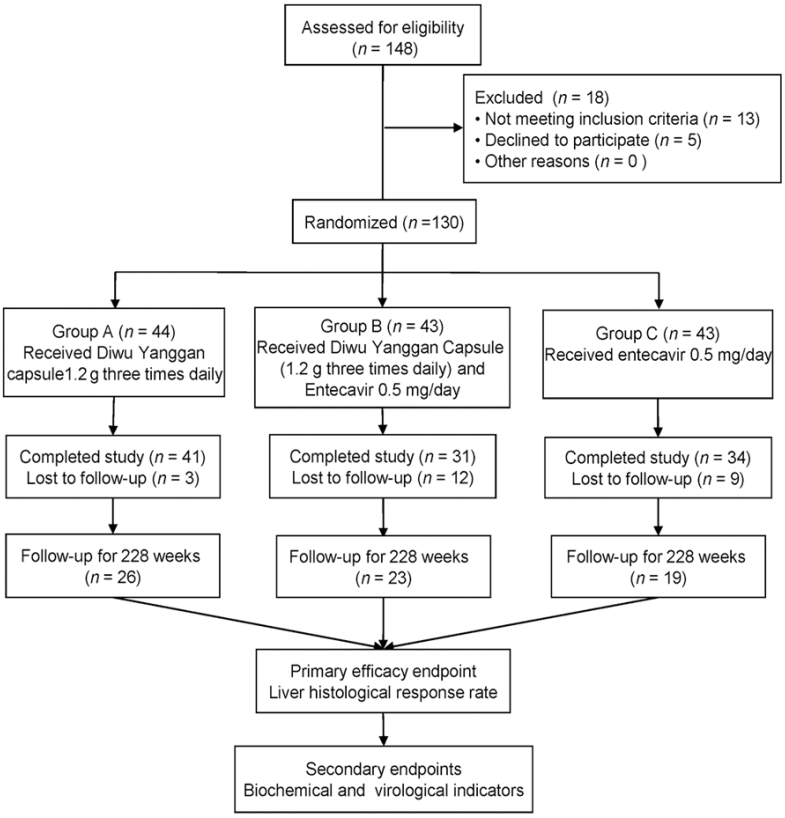

Figure 1. Trial flow diagram.

ogy, clinical features, prognosis and treatment strategies [6, 7]. Compared with HBeAg-positive CHB patients, those with HBeAg-negative CHB show the following characteristics: disease duration is relatively long; infected individuals are relatively older; it is more common in males; individuals often have intermittent or persistent viral replication; precore/core promoter mutations of HBV are common; serum alanine aminotransferase (ALT) and HBV DNA levels often fluctuate; spontaneous reduction of disease is less common; severe liver necrosis, inflammation and progressive liver fibrosis are common; the effect of antiviral drug treatment for HBeAg-positive CHB is relatively poor due to the low liver tissue response rate and high risk of recurrence after drug cessation. With the gradual increase in the course of disease, the risk of liver failure, liver cirrhosis and liver cancer has gradually increased, which has become a new clinical treatment problem [8-16].

Previous experiments and clinical studies have shown that Diwu Yanggan capsule has the ability to inhibit HBV replication, reduce liver damage and anti-liver fibrosis and regulate immunity and liver regeneration, thereby reducing the risk of liver cancer in patients with HBeAg-negative CHB. Based on preliminary research, we used randomized controlled clinical trials to observe the clinical efficacy and safety of Diwu Yanggan capsule in the treatment of HBeAg-negative CHB, which provides evidence for the treatment of HBeAg-negative CHB using Diwu Yanggan capsule alone or combined with an antiviral drug.

Materials and methods

Study design and population

Since January 2011, we have conducted a non-blind, randomized controlled clinical trial in patients with HBeAg-negative CHB. All patients were recruited from the Hubei Provincial Hospital of TCM. The registration number of the Chinese Clinical Trial Registry is ChiCTR-TRC-12002962, to be found at http://www.chictr.org.cn/searchproj.aspx. The Ethics Committee of Hubei Province Hospital of Traditional Chinese Medicine reviewed (approval number, HBZY2010-C01) and approved the protocol. All participants provided their written informed consent prior to enrollment.

Inclusion criteria were the following: age 18-65 years; patients with a history of hepatitis B virus infection or who were HBsAg-positive for more than 6 months and HBsAg-positive and/or Hepatitis B Virus (HBV) DNA-positive; patients with HBeAg-negative, HBV DNA-positive CHB and mildly abnormal ALT levels (< 2 × ULN) or with HBeAg-negative, HBV DNA-negative CHB and normal/mild ALT levels; patients with significant inflammatory necrosis (liver inflammation grade ≥ G2) or significant fibrosis (liver fibrosis grade fibrosis ≥ S2) [17].

Exclusion criteria were the following: patients with hepatitis B virus infection concurrent with

Table 1. Baseline characteristics of patients *n* (%)

Characteristics	Group A (*n* = 44) DWYG	Group B (*n* = 43) DWYG + Entecavir	Group C (*n* = 43) Entecavir	*P* value
Gender, Male	28 (63.64)	33 (76.74)	32 (74.42)	0.350
Age, mean ± SD, yr	40.09 ± 12.98	40.84 ± 9.68	37.53 ± 11.22	0.372
Course of disease, yr	17.75 ± 7.41	19.72 ± 7.76	20.42 ± 8.93	0.279
Liver histopathology				
G = 2	21/24 (87.50%)	19/20 (95.00%)	15/16 (93.75%)	0.725
S = 2	11/24 (45.83%)	16/20 (80.00%)	11/16 (68.75%)	0.063
Biochemistry				
ALT (U/L)	33.78 ± 15.27	38.56 ± 18.92	40.19 ± 19.81	0.231
AST (U/L)	27.53 ± 8.17	42.02 ± 64.48	33.21 ± 12.69	0.207
GGT (U/L)	21.66 ± 14.05	31.97 ± 25.85	28.05 ± 21.96	0.064
ALP (U/L)	64.20 ± 18.63	71.62 ± 20.14	74.57 ± 22.78 (*n* = 42)	0.057
TP (g/L)	72.22 ± 4.60	73.76 ± 3.90	73.68 ± 5.60 (*n* = 42)	0.238
ALB (g/L)	43.61 ± 3.08	43.88 ± 3.00	45.00 ± 3.47 (*n* = 42)	0.109
GLOB (g/L)	28.61 ± 3.48	29.85 ± 3.42	28.71 ± 4.23 (*n* = 42)	0.230
TBIL (μmol/L)	13.59 ± 5.58	14.12 ± 10.38	13.75 ± 4.85	0.942
DBIL (μmol/L)	3.29 ± 1.75	3.58 ± 2.94	3.50 ± 1.53	0.810
IBIL (μmol/L)	10.30 ± 4.39	10.54 ± 7.79	10.25 ± 3.82	0.968
Virology				
HBV DNA, $\log_{10}$ IU/ml	3.38 ± 0.91	3.46 ± 1.12	3.49 ± 1.23	0.897
HBsAg, $\log_{10}$ IU/ml	2.46 ± 0.74	2.64 ± 0.67	2.73 ± 0.65	0.159

other types of viral hepatitis; hepatitis caused by drug or alcohol intoxication; patients with autoimmune hepatitis; patients who were diagnosed with liver failure, liver cirrhosis or liver cancer; patients complicated by other severe systematic diseases or psychiatric diseases; patients who were lactating or pregnant; patients with drug allergy.

Intervention

All patients diagnosed with HBeAg-negative CHB were randomly assigned to three different treatment groups at a ratio of 1:1:1 using a computer-based random number generation program. The program was designed and used by a statistician from Hubei University of Chinese Medicine. And the sample size was determined based on past clinical efficacy. The patients in group A were treated with Diwu Yanggan capsule (A new drug of traditional Chinese medicine, Hubei Food and Drug Administration Approval No.Z20113160, China) 1.2 g three times daily. The patients in group B were treated with Diwu Yanggan capsule 1.2 g three times daily in combination with entecavir dispersible tables (entecavir) 0.5 mg/day (National Drug Approval No.H20052237, Bristol-Myers Squibb Co., Ltd., China) orally. The patients in group C were treated with entecavir 0.5 mg/day orally [18, 19]. Diwu Yanggan capsule is a traditional Chinese medicine preparation and is mainly composed of the following traditional Chinese herbal medicine components: shudihuang (Rehmanniae Radix Praeparata) (20%), yinchen (Artemisiae Scopariae Herba) (33.2%), jianghuang (Curcumae Longae Rhizoma) (13.4%), wuweizi (Schisandrae Chinensis Fructus) (20%) and gancao (Glycyrrhizae Radix et Rhizoma) (13.4%). Its preparation techniques were stable, and the quality was controlled.

Outcome measures

The primary efficacy endpoint was the liver histological response rate in each group. biochemical (ALT, AST, GGT, ALP, TP, ALB, GLOB, TBIL, DBIL, IBIL, HBV DNA, HBsAg) and virological responses (HBV DNA, HBsAg) served as secondary endpoints. BECKMAN COULTER CHEMISTRY ANALYZER AU5800 and ancillary reagents were used to measure biochemical responses. HBV DNA and HBsAg levels were measured

Table 2. Biochemical and virological responses after 24-wk treatment

	Group A (*n* = 36) DWYG	Group B (*n* = 28) DWYG + Entecavir	Group C (*n* = 27) Entecavir	*P* value
Biochemistry				
ALT (U/L)	26.81 ± 14.86	27.07 ± 14.80	29.41 ± 19.08	0.799
AST (U/L)	24.50 ± 7.97	25.57 ± 10.00	26.93 ± 13.51	0.663
GGT (U/L)	21.22 ± 13.23	25.82 ± 14.21	24.93 ± 16.19	0.400
ALP (U/L)	59.31 ± 15.97	66.96 ± 22.89 (*n* = 27)	69.04 ± 19.80	0.112
TP (g/L)	72.62 ± 3.73	74.89 ± 5.27	75.04 ± 4.97	0.066
ALB (g/L)	44.29 ± 2.90	45.74 ± 3.32	45.32 ± 3.16	0.160
GLOB (g/L)	28.34 ± 3.27	29.16 ± 3.14	29.72 ± 3.65	0.264
TBIL (μmol/L)	12.33 ± 5.58	13.24 ± 8.77	12.89 ± 4.47	0.852
DBIL (μmol/L)	3.65 ± 2.69	3.57 ± 2.28 (*n* = 27)	3.49 ± 1.60	0.959
IBIL (μmol/L)	8.68 ± 3.64	9.69 ± 7.88 (*n* = 27)	9.37 ± 3.61	0.739
Virology				
HBV DNA, $\log_{10}$ IU/ml	3.07 ± 0.59 (*n* = 24)	2.95 ± 0.90 (*n* = 21)	3.09 ± 1.16 (*n* = 22)	0.851

Some patients did not receive the test at the time point but after a period of time to accept the test is not included in the shedding.

respectively by ABI ViiA™ 7 and ROCHE Modular Analytics E170 with the corresponding reagents. Patients were evaluated for biochemical and virological responses at baseline and 48 wk of treatment. The liver histological response rate was evaluated at wk 48. The incidence of liver cirrhosis, biochemical and virological responses was observed during 228 wk of follow-up. Liver biopsy specimens were collected by doctors in the liver disease department, and the requirements of the liver tissue were 15-20 mm. Serial sections of all liver tissues were stained with hematoxylin-eosin (H&E) and Masson staining. The liver histological response was defined by an HAI fibrosis score (S1-S4) that was reduced by one grade or more, while the inflammatory score (G1-G4) did not increase or decrease [17, 20]. All tests were performed by the Departments of Pathology and Laboratory Medicine of Hubei Provincial Hospital of TCM. The incidence of liver cirrhosis and biochemical and virological responses was detected in each group during the 228-wk follow-up period.

Statistical analysis

The statistical analysis was performed using SPSS19.0 (IBM Corp, Armonk, NY, USA) and Graphpad Prism 6 (GraphPad Software Inc., La Jolla, CA, USA). The data were analyzed by X^2 or Fisher test, and comparisons among groups were analyzed using one-way ANOVA. Mantel-Cox statistics were used to analyze the degree of pathology. A $P < 0.05$ was considered significant.

Results

The trial ran from January 2011 to September 2016. In total, 130 participants were randomized and allocated to treatment. All participants were treated for 48 wk and then followed up to 228 wk after their final treatment (**Figure 1**). Entecavir administration was continued during follow-up. A total of 24 patients did not complete the study; therefore, 106 cases were included in the final statistical analysis. The patients in the three groups showed similar characteristics at baseline (**Table 1**).

Liver histological responses

During the treatment of HBeAg-negative chronic hepatitis B cases, the liver histological response was better with DWYG alone or DWYG combined with entecavir group than that of the positive control, the entecavir treatment group. Liver biopsy pathological changes in each group before and after treatment were observed by H&E and Masson staining.

After 48 wk of treatment, the histological response rates in patients with Diwu Yanggan capsule and entecavir were 54.55% and 22.22%, respectively (**Table 3**). There was no significant difference between the two groups ($P > 0.05$). The liver histology response rate in

Table 3. Biochemical and virological responses after 48-wk treatment

	Group A (*n* = 35) DWYG	Group B (*n* = 29) DWYG + Entecavir	Group C (*n* = 25) Entecavir	*P* value
Liver histological response rate (%)	50.00 (5/10)	71.43 (10/14)[a]	22.22 (2/9)	0.073
Biochemistry				
ALT (U/L)	30.78 ± 23.47	26.52 ± 16.71	27.36 ± 12.68	0.632
AST (U/L)	27.00 ± 12.14	23.03 ± 6.10	24.40 ± 6.34	0.208
GGT (U/L)	25.43 ± 20.22	25.52 ± 15.46	25.96 ± 13.82	0.992
ALP (U/L)	67.34 ± 20.80	77.97 ± 24.54	75.28 ± 21.89	0.147
TP (g/L)	75.49 ± 3.76	76.29 ± 3.77	75.87 ± 5.80	0.770
ALB (g/L)	45.92 ± 2.94	47.27 ± 2.46	46.82 ± 3.01	0.152
GLOB (g/L)	29.57 ± 3.33	29.02 ± 3.23	29.05 ± 4.15	0.787
TBIL (μmol/L)	12.11 ± 6.79	12.04 ± 6.76 (n = 28)	13.78 ± 5.95	0.549
DBIL (μmol/L)	3.94 ± 2.37	3.86 ± 1.35 (n = 28)	4.20 ± 1.75	0.797
IBIL (μmol/L)	8.17 ± 4.62	8.19 ± 5.93 (n = 28)	9.58 ± 4.49	0.502
Virology				
HBV DNA, $\log_{10}$ IU/ml	3.01 ± 0.49 (*n* = 27)	2.78 ± 0.13 (*n* = 24)	2.90 ± 0.53 (*n* = 18)	0.152
HBsAg, $\log_{10}$ IU/ml	2.73 ± 0.78 (*n* = 28)	2.82 ± 0.54 (*n* = 27)	2.82 ± 0.50 (*n* = 21)	0.839

[a]Group B *vs* Group C, *P* = 0.036.

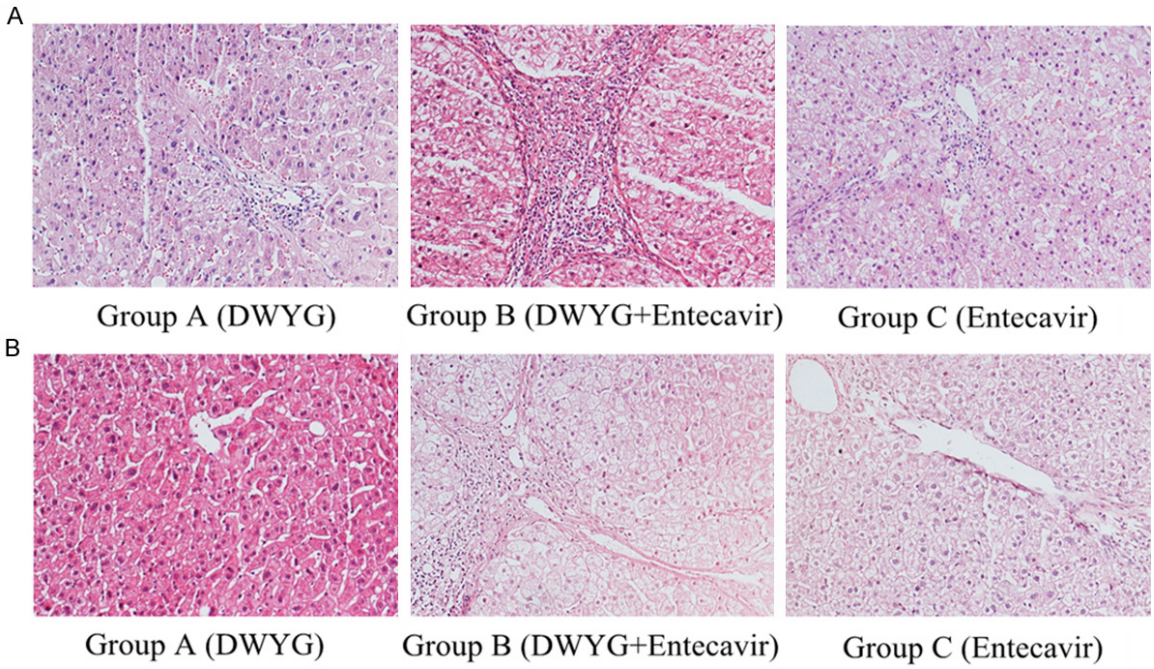

Figure 2. Liver histological examination of each group at baseline and after 48-wk treatment (Hematoxylin-eosin staining, 100 ×). A, B. Liver tissue pathological changes of patients with HbeAg-negative CHB at baseline and after 48-wk treatment.

the group taking Diwu Yanggan capsule in combination with entecavir was 71.43%; this difference was significant compared with that seen in the entecavir treatment group (71.43% *vs* 22.22%; *P* = 0.036). The pathology images of the liver biopsy are shown in **Figures 2** and **3**. Pathological observation of the liver issue showed the following: pathological changes, such as hepatic cell swelling, inflammatory cell infiltration, fatty degeneration, necrosis, focal

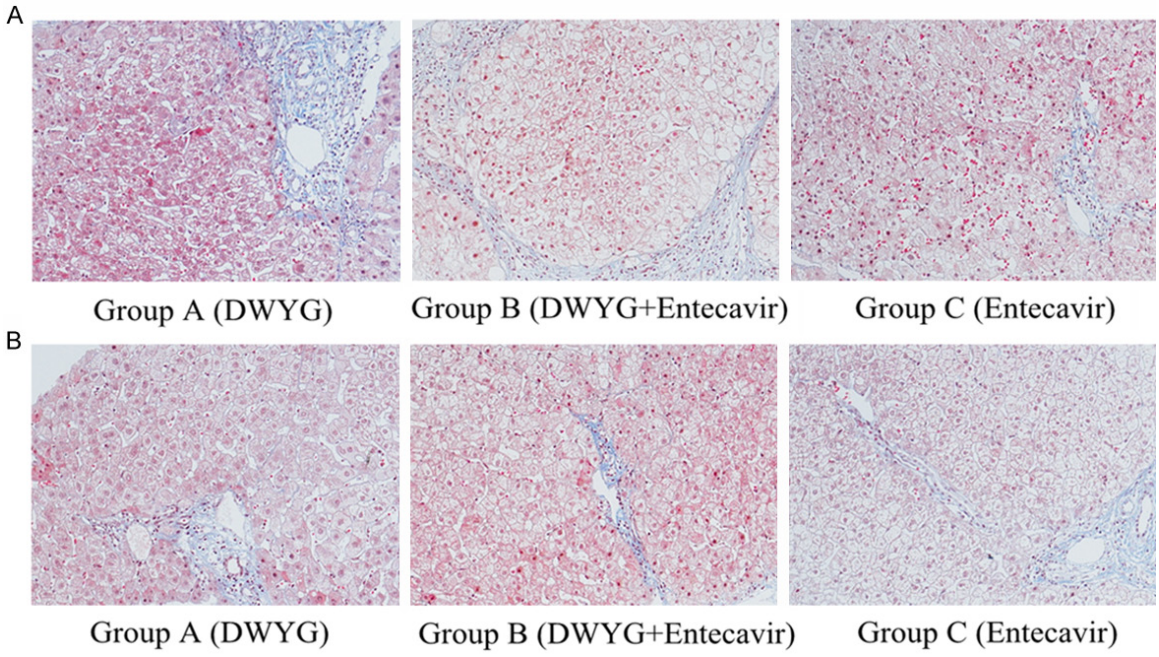

Figure 3. Liver histological examination of each group at baseline and after 48-wk treatment (Masson staining, 100 ×). A, B. Liver tissue pathological changes of patients with HbeAg-negative CHB at baseline and after 48-wk treatment.

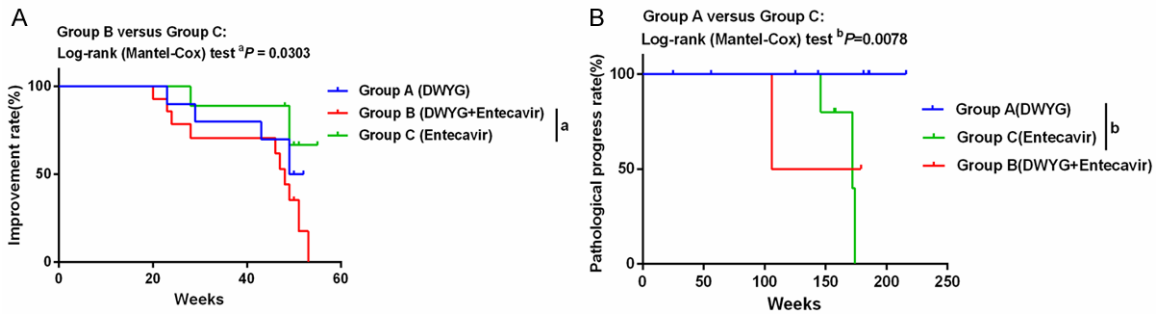

Figure 4. Survival curves during treatment and follow-up. A. Liver histology improvement rates were recorded after 48-wk treatment and analyzed by Log-rank (Mantel-Cox) test. $^{a}P < 0.05$. B. Pathological progression rates during 228 wk of follow-up were recorded and analyzed by Log-rank (Mantel-Cox) test. $^{b}P < 0.01$.

necrosis and piecemeal necrosis, and periportal fibrous tissue hyperplasia were observed after 48 wk of treatment with entecavir (control group). Pathological changes lessened significantly after DWYG alone or when combined with entecavir. In the survival curve analysis, Mantel-Cox showed that the improvement time of liver tissues was much earlier in the DWYG combined with entecavir treatment group than those in the DWYG alone or entecavir alone group. The liver improved more in the DWYG combined with entecavir treatment group than in the entecavir group; this difference was significant ($P = 0.0303$) (**Figure 4A**).

Biochemical and virological responses

The biochemical response of HBeAg-negative chronic hepatitis B was similar among the DWYG alone, DWYG combined with entecavir and entecavir alone groups.

The biochemical results (ALT, AST, GGT, ALP, TP, ALB, GLOB, TBIL, DBIL, IBIL) at 24 and 48 wk of treatment were not significantly different among the three groups ($P > 0.05$; **Tables 2** and **3**).

The virological response to DWYG was similar to the other two groups in the treatment of

Table 4. Incidence of liver cirrhosis, biochemical and virological responses during 228-wk follow-up

	Group A (*n* = 26) DWYG	Group B (*n* = 23) DWYG + Entecavir	Group C (*n* = 19) Entecavir	*P* value
Incidence of liver cirrhosis (%)	0.00 (0/9)[a]	50.00 (1/2)	60.00 (3/5)	0.019
Biochemistry				
ALT (U/L)	22.12 ± 10.33	22.17 ± 7.73	27.63 ± 13.95	0.175
AST (U/L)	21.31 ± 5.53	20.52 ± 4.54	23.58 ± 6.47	0.191
GGT (U/L)	25.28 ± 19.17 (*n* = 25)	22.18 ± 11.94 (*n* = 22)	26.32 ± 13.40	0.665
ALP (U/L)	67.68 ± 19.20 (*n* = 25)	71.77 ± 18.49 (*n* = 22)	72.74 ± 26.30	0.696
TP (g/L)	76.12 ± 3.52	77.08 ± 5.24	76.01 ± 3.65	0.647
ALB (g/L)	46.04 ± 3.15	47.08 ± 3.03	46.69 ± 3.11	0.497
GLOB (g/L)	30.07 ± 3.47	30.00 ± 2.95	29.32 ± 3.97	0.745
TBIL (μmol/L)	14.69 ± 8.55	11.92 ± 4.91 (*n* = 22)	13.42 ± 5.06	0.898
DBIL (μmol/L)	4.67 ± 2.92	3.78 ± 1.56 (*n* = 22)	4.23 ± 1.91	0.570
IBIL (μmol/L)	10.03 ± 5.77	7.82 ± 3.88 (*n* = 22)	9.18 ± 3.68	0.898
Virology				
HBV DNA, $\log_{10}$ IU/ml	2.43 ± 0.90 (*n* = 21)	2.37 ± 0.55 (*n* = 12)	2.37 ± 1.10 (*n* = 10)	0.969
HBsAg, $\log_{10}$ IU/ml	2.83 ± 0.69 (*n* = 21)	2.70 ± 1.28 (*n* = 14)	2.91 ± 0.53 (*n* = 14)	0.805

[a]Group A *vs* Group C, *P* = 0.027.

HBeAg-negative chronic hepatitis B at 48 wk of treatment (*P* > 0.05; **Table 3**).

Follow-up

The incidence of liver cirrhosis, virological and biochemical responses was observed during 228 wk of follow-up. The improved HAI Ishak system was used as the evaluation criteria for the degree of liver pathology [20]. Liver cirrhosis was diagnosed with significant bridging fibrosis, bridging necrosis and nodule formation (pseudolobuli). Moreover, a reduction in the incidence of liver cirrhosis was the expected target during follow-up.

The results showed that the difference in the incidence rate of liver cirrhosis among the three groups was significant (*P* = 0.019). The difference was significant in the DWYG group compared with the entecavir group (0% *vs* 60%; *P* = 0.027).

In addition to the pathological changes such as hepatic cell swelling, inflammatory cell infiltration, fatty degeneration, necrosis, focal necrosis and piecemeal necrosis, and periportal fibrous tissue hyperplasia during follow-up, 60% of patients also had widening of the hepatic portal area, bridging necrosis, bridging fibrosis and pseudolobule formation or nodules. The patients treated with DWYG had no cirrhosis, nodule formation or pseudolobules.

The Log-rank (Mantel-Cox) survival curve analysis showed that the DWYG treatment group slowed liver disease progression compared with that seen in the entecavir group. The difference was significant (*P* = 0.0078) (**Figure 4B**). There were no significant differences in virological and biochemical responses among the three groups (*P* > 0.05; **Table 4**).

Safety

Eight cases in our study experienced abdominal distention, anorexia and other mild gastrointestinal reactions, which reduced or disappeared spontaneously and did not affect the continuation of treatment. No significant adverse events occurred.

Discussion

At present, there are three types of measures to treat chronic hepatitis B: liver tissue response, viral response and biochemical responses, in which liver tissue response is the “gold standard”.

In the past, substantial attention has been focused on HBeAg seroconversion and HBeAb positive conversion (“seroconversion”) for chronic hepatitis B (CHB) treatment, and seroconversion is used as a main assessment indicator for curative effect judgment. However, patients with “seroconversion” are actually

patients with HBeAg-positive CHB that has changed to HBeAg-negative CHB. In patients with HBeAg-negative CHB, replication of HBV mutant strains, as well as other adverse factors, leads to the development of disease recurrence or dormancy, such that the patient's condition is unable to improve by itself [21]. Studies have found that HBeAg-negative CHB is difficult to treat because the tissue damage is greater than in HBeAg-positive CHB, and antiviral therapy drugs cannot effectively improve the histological damage and prevent the progression of disease. Therefore, the risk of liver failure, liver cirrhosis, liver cancer and other serious outcomes still increases annually [22]. In this clinical trial, we found that the vast majority (91.67%) of HBeAg-negative CHB patients, regardless of normal HBV DNA, ALT and AST, had liver histology inflammatory or fibrosis scores that reached grade 2 or above. In the study, the liver histological response rate was only 22.22% after 48 wk of treatment with entecavir, and the incidence rate of liver cirrhosis was 60.00% during 228 wk of follow-up. The progression of CHB depends on the interaction between HBV and the host; there is no chronic hepatitis B without HBV. However, HBV cannot completely determine the fate of chronic hepatitis B, in which the body's host factors playa very important role [23, 24]. The results indicated that the liver histologic response rate of patients with entecavir was not high, and it failed to completely block the progression of disease. Because of the strong, low rates of resistance to HBV antivirals, the most widely used clinical Chinese entecavir was used as a positive control. A possible reason is that HBV replication in patients with HBeAg-negative CHB is not the primary mechanism of disease progression; host factor disorders (liver regeneration repair mechanism, immune function, genetic background, etc.) will promote the main contradictions of disease progress, and antiviral drugs have a weaker effect on host factors.

Most of the existing research and treatment of chronic hepatitis B has focused on viral factors, such as viral load, virus genotype, virus mutation and the relationship between drug resistance and prognosis. However, studies on host factors are relatively rare. HBV DNA replication in patients with HBeAg-negative CHB is generally low. Antiviral treatment inhibits HBV DNA replication but has little effect on liver histological response, limiting its function to stop disease progression. Our previous study found that the mechanisms of liver regeneration and repair are the important factors that affect the progression of HBeAg-negative CHB. Specifically, the normal mechanism of liver regeneration and repair promotes HBeAg-negative CHB in stable conditions or tends to cause recovery. In contrast, the abnormal mechanism of liver regeneration and repair can promote recurrence or deterioration of HBeAg-negative CHB [25, 26].

Preliminary clinical and experimental studies have shown that Diwu Yanggan capsule inhibits HBV replication, anti-liver injury and immune regulation but also regulates liver regeneration. Our data showed that it improved normal liver regeneration to repair liver tissue injury and inhibited abnormal liver regeneration to lessen hepatic fibrosis and hepatic precancerous lesions of rats [18, 19, 27-34]. This clinical study found that the biochemical and virological responses to DWYG alone were comparable to entecavir treatment. DWYG improves the response rate of liver function by influencing host factors (including regulation of liver regeneration, liver damage and immune regulation). The histological response standard of liver tissue in the treatment period was represented by a decrease in the HAI fibrosis score, and the inflammation score did not change. In the follow-up period, lessening of hepatic cirrhosis was the primary efficacy criterion. Compared with the viral response after 48 wk of treatment in the single DWYG drug or DWYG combined with entecavir treatment groups, the hepatic histological response was better in the entecavir treatment alone group. The degree of liver pathology was significantly reduced compared with that seen with entecavir treatment alone. Differences were significant in the survival curves ($P = 0.036$). Log-rank (Mantel-Cox) analysis showed that the liver histology improvement in the DWYG combined with entecavir treatment group was better than that in the control group with entecavir, and the difference was significant ($P = 0.0303$). The survival curve at follow-up showed that the incidence of liver cirrhosis in the DWYG combined with entecavir treatment group in patients with HBeAg-negative CHB was significantly lower than that of the entecavir group (0% *vs* 60%), and the difference was significant ($P = 0.0078$). The sur-

vival curve showed that the pathological progression of liver streated with DWYG was slower than that of the entecavir treatment group (control group), and the rate of liver cirrhosis significantly decreased.

Previous studies of traditional Chinese medicine in the treatment of HBeAg-negative CHB have primarily used medicines based on antiviral drugs, and synergy between traditional Chinese and Western medicine has been observed [35-37].

In conclusion, the clinical study results suggest that using DWYG alone can improve the liver histological response rate of patients with HBeAg-negative CHB. Specifically, it has a stable long-term curative effect and can significantly reduce the incidence of liver cirrhosis. In addition to using antiviral drugs, treating HBeAg-negative CHB with DWYG is also an important way to improve the clinical effect by influencing host factors, which provides a reference for further research and clinical applications. Moreover, because of the particularity of traditional Chinese medicine preparation, this trial is a non-blind trial, not entirely eliminate the possibility of trial bias, it is necessary for multi-center, large sample studies.

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Disclosure of conflict of interest

None.

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