Entecavir add-on Peg-interferon therapy plays a positive role in reversing hepatic fibrosis in treatment-naïve chronic hepatitis B patients: a prospective and randomized controlled trial

Jing-Mao Yang[1], Li-Ping Chen[2], Ya-Jie Wang[3], Bei Lyu[4], Hong Zhao[5], Zhi-Yin Shang[1], Jun Li[1], Zhen-Yu Fan[1], Sheng-Di Wu[4], Xiao Ming[1], Xian Li[1], Shao-Ping Huang[1], Ji-Lin Cheng[1]

[1]Department of Gastroenterology and Hepatology, Shanghai Public Health Clinical Center, Fudan University, Shanghai 201500, China;
[2]Shanghai Medical College of Fudan University, Shanghai 200032, China;
[3]Department of Gastroenterology and Hepatology, Jinshan Hospital, Fudan University, Shanghai 201500, China;
[4]Department of Gastroenterology and Hepatology, Zhongshan Hospital, Fudan University, Shanghai 200032, China;
[5]Department of Gastroenterology and Hepatology, Dongyang People's Hospital, Dongyang, Zhejiang 322100, China.

Abstract

Background: The efficacy of entecavir (ETV) add-on peg-interferon therapy compared with ETV monotherapy in treatment-naïve hepatitis B virus (HBV) patients remains controversial. We investigated whether adding peg-interferon to ongoing ETV treatment leads to a better curative effect or not.

Methods: All patients have been recruited between August 2013 and January 2015 from the Shanghai Public Health Clinical Center and Zhongshan Hospital (China). Eligible HBV patients ($n = 144$) were randomly divided (1:1) to receive either ETV monotherapy ($n = 70$) or peg-interferon add-on therapy from week 26 to 52 ($n = 74$). Patients were followed-up for at least 2 years. Indexes including hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) seroconversion rate, sustained virologic response, transient elastography value, and histological scores were evaluated every 3 months until the end of the study. The rate of patients with HBsAg loss was defined as the primary endpoint criteria.

Results: At week 26, no patient achieved HBsAg seroconversion in either group. At week 52, one patient in the monotherapy group was HBsAg-negative but there was none in the combination therapy group. The monotherapy group showed significantly better liver function recovery results than the combination therapy group. At week 78, one patient in the combination group had HBsAg seroconverted. At week 104, only three patients in the combination therapy group were HBsAg-negative compared with one patient in monotherapy. The mean alanine aminotransferase and aspartate aminotransferase levels and transient elastography values decreased significantly compared with baseline. Both groups showed a favorable decrease in alpha-fetoprotein (monotherapy: 4.5 [2.8, 7.1] *vs.* 2.2 [1.8, 3.1] ng/mL, $P < 0.001$; combination therapy: 5.7 [3.0, 18.8] *vs.* 3.2 [2.0, 4.3] ng/mL, $P < 0.001$) and an improved result of liver biopsy examination scores. The combination group showed a better improvement in histology compared with the monotherapy group (mean transient elastography value 6.6 [4.9, 9.8] *vs.* 7.8 [5.4, 11.1] kPa, $P = 0.028$). But there was no significant difference in HBsAg conversion rate (1.8% [1/56] *vs.* 4.1% [3/73], $P = 0.809$) and HBeAg conversion rate (12.5% [7/56] *vs.* 11.0% [8/73], $P = 0.787$), as well as HBV-DNA, sustained virologic response (93.2% *vs.* 98.5%, $P = 0.150$) between the two groups.

Conclusions: Both therapies supported liver function recovery and histology improvement. Combination therapy did not show better anti-viral efficacy in HBsAg or HBeAg seroconversion compared with monotherapy. However, combination therapy played a more positive role in reversing hepatic fibrosis compared with monotherapy.

Trial registration: ClinicalTrials.gov: NCT02849132; https://clinicaltrials.gov/ct2/show/NCT02849132

Keywords: Peg-interferon; Entecavir; Chronic hepatitis B; Curative effect; Combination therapy

Introduction

There are approximately 257 million patients infected with hepatitis B virus (HBV) worldwide, which represents a challenge to public health despite the clinical application of new drugs and efficacious vaccines.[1] Nucleos(t)ide analogs (NAs) are used more commonly in HBV treatment protocols because they can suppress HBV-DNA replication, help to recover liver function, improve histology, and

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Jing-Mao Yang and Li-Ping Chen contributed equally to this work.

Correspondence to: Dr. Ji-Lin Cheng, Department of Gastroenterology and Hepatology, Shanghai Public Health Clinical Center, Fudan University, Caolang Road 2901, Shanghai 201500, China
E-Mail: 2360931679@qq.com

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obtain a functional cure which defined as hepatitis B surface antigen (HBsAg)-negative regardless of the presence or absence of hepatitis B surface antibody (HBsAb). Peg-interferon (IFN)α-2a is an immune-modulating drug that can induce cytotoxic T-cells, which clears the HBV virus from infected cells through the immunomodulatory pathway and reduces the covalently closed circular DNA levels.[2,3] Peg-IFNα-2a was shown to achieve a higher rate of HBeAg seroconversion and HBsAg seroclearance compared with entecavir (ETV) treatment.[4,5] Combination therapy of lamivudine and peg-IFNα-2a showed a higher virologic response rate but there was no improvement in the post-therapy response compared with monotherapy.[6] However, peg-IFNα-2a has several disadvantages including a moderate anti-viral effect, sustained risk of adverse events, inferior tolerability, and subcutaneous injections. What's more, it requires regular clinical follow-up visits and a strict administration schedule. Therefore, NAs and peg-IFNα-2a combination therapy have been carried out to find an optimal efficacy, but the results of these trials are controversial because of the varying designs and evaluation criteria.[7-9] Some studies considered that combination therapy was better than monotherapy, but anti-viral therapeutic options are largely influenced by the cost of drugs in China.[10] Most treatment-naïve patients generally start on NAs as an initial treatment because they are inexpensive and have a confirmed curative effect despite drug resistance.

Therefore, we designed this prospective trial to evaluate the curative effect of adding peg-IFN to ongoing ETV therapy in treatment-naïve patients. Given the side effects of peg-IFNα-2a monotherapy and drug-resistance of ETV long-term therapy, we evaluated whether shortening the add-on course (26 weeks) of peg-IFNα-2a improve outcomes such as enhancing virologic response and sustained suppression of HBV DNA or not.

Methods

Ethics approval

This study was conducted in compliance with the *Declaration of Helsinki* and approved by the Ethical Committee of Shanghai Public Health Clinical Center (No. 2016-S026-04). All patients signed an informed consent form indicating that their participation was voluntary and their samples could be used for research. They were also informed of adverse events.

Study design

All patients have been recruited between August 2013 and January 2015 from the Shanghai Public Health Clinical Center and Zhongshan Hospital (China). Eligible participants were randomly divided into two groups at the start of the study by using a computerized randomization program. One hundred and forty-four eligible patients were randomly divided, in a 1:1 ratio, to receive either ETV monotherapy ($n = 70$) or Peg-IFN add-on therapy ($n = 74$). Both groups were given ETV for 2 years, but after 26 weeks, the combination therapy group received additional peg-IFNα-2a once weekly from week 26 to 52. The efficacy of NAs monotherapy and combination therapy was compared every 3 months until the end of the study. Liver histology was also evaluated when participants were enrolled in the trial and after 2 years of therapy. They underwent a liver biopsy at the beginning and end of the study.

Inclusion and exclusion criteria

All chronic HBV patients were positive for HBsAg and had an HBV-DNA viral load >500 U/mL with two consecutive tests at least 6 months apart, and the patients were treatment-naïve. Patients who were either HBeAg-positive or negative were included. Exclusion criteria were as follows: (1) co-infected with hepatitis A virus, hepatitis C virus, hepatitis D virus, or human immunodeficiency virus; (2) decompensated liver disease (such as hepatocyte dysfunction and portal hypertension) or a history of esophageal varices; (3) hepatocellular carcinoma; (4) pregnancy or lactation, or liver transplantation; (5) alcoholic hepatitis, drug hepatitis, autoimmune disease, or metabolic liver disease; (6) comorbidities such as diabetes mellitus, arterial hypertension, dyslipidemia, coronary artery diseases, and thyropathy. The trial design is shown in Figure 1.

Measurements

Before participants were enrolled in the trial, laboratory tests and routine examinations were performed at 3-month intervals for 6 months. Serum HBsAg and HBeAg levels were tested by Architect i2000 analyzer (Abbott Diagnostics, Abbott Park, IL, USA; HBsAg range of 0.05–52,000 U/mL and HBeAg cut-off index <10 U/mL), HBV DNA was amplified using the iCycler device (Bio-Rad, Berkeley, California, USA; lower limit of quantification: 500 U/mL). Liver biopsies were assessed at the Zhongshan Hospital and Shanghai Public Health Clinical Center, Fudan University, Shanghai, China.

Evaluation criteria

HBsAg/HBeAg, serum HBV-DNA viral load, and liver function were assessed at the beginning of treatment. The rate of patients with HBsAg loss was defined as the primary endpoint criteria. The proportion of patients with sustained suppression of HBV DNA below 500 IU/mL, HBeAg seroconversion loss or seroconversion, histology improvement, and liver function were defined as secondary endpoints. HBsAg loss refers to undetectable serum HBsAg <0.05 U/mL, and HBsAg/HBeAg seroconversion means the detectable HBeAb/HBsAb antibodies. HBV-DNA <500 U/mL was defined as an undetectable level. Ishak score was evaluated by a professional pathologist. A reduction in the Ishak score or transient elastography measurement between the first day of treatment and the end of therapy was defined as the histology improvement criterion. The liver cirrhosis diagnosis was based on pathological stages and grades of liver histology and transient elastography standard (liver stiffness >12.4 kPa in chronic hepatitis B patients with normal bilirubin and 1 × upper limit of normal (ULN) < alanine aminotransferase

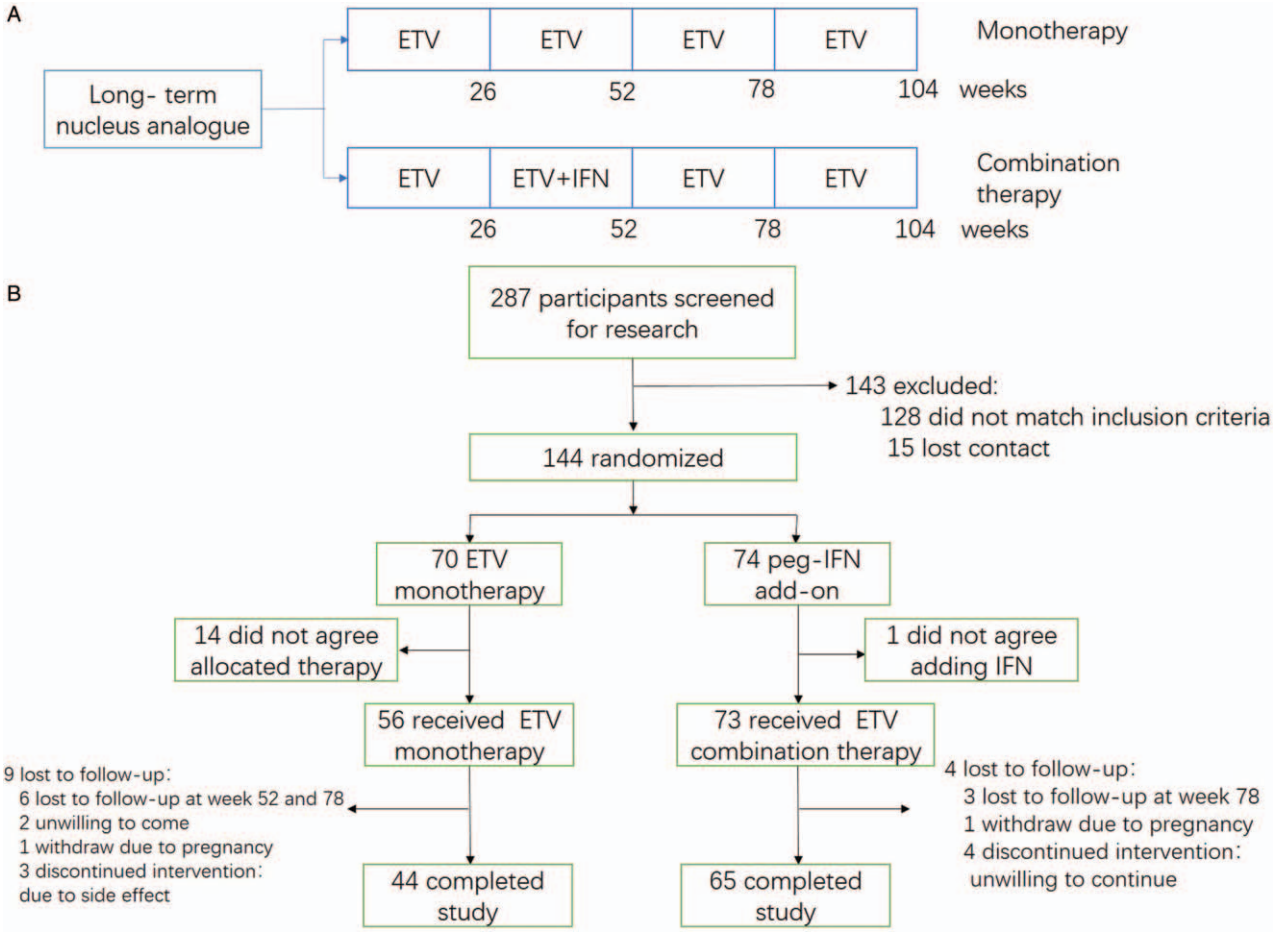

Figure 1: The trial design and flow chart. (A) Trial design. Combination therapy adding on pre-IFN-α-2a from week 26 to 52. All patients were followed-up at least for 104 weeks. (B) Flowchart showing the disposition of patients during the research. ETV: entecavir; IFN: Interferon.

(ALT) < 2 × ULN considered the progression of liver fibrosis).[11] We analyzed HBsAg/HBeAg loss or seroconversion, viral load, and liver function every 3 months and the end of treatment between the two groups. Adverse events were also recorded in both groups.

Statistical analysis

According to the method of sample size estimation in the randomized controlled trial, the ratio of sample size between the two groups was 1:1, and the predicted rate of reversal liver fibrosis was 63% and 83%, respectively. With the use of bilateral test, the test level was equal to 0.05, β as 0.2, and the sample size of the cohort was expected to be 70 people in each group. All statistical analyses were processed using SPSS version 19 (SPSS Inc. Chicago, IL, USA). The continuous data were presented as the mean ± standard deviation, median and interquartile range, as appropriate. Continuous variables were compared by the Student's *t* test or Mann-Whitney *U* test. And categorical data were presented as *n* (%) compared by the Chi-square test or Fisher exact test. The HBsAg/HBeAg seroconversion rate between the two groups was compared using the Chi-square test. *P* value of <0.05 was defined as statistically significant.

Results

Baseline characteristics

All baseline characteristics of the two groups are shown in Table 1. Almost all clinical data from the two groups were comparable. All patients were HBsAg positive, with an HBV viral load >500 IU/mL, no participant had decompensated liver cirrhosis. At the beginning of the study, some patients showed high level of alpha-fetoprotein (AFP), but computed tomography (CT) or magnetic resonance imaging (MRI) results showed no abnormal mass or cancer in the liver.

Clinical efficacy of two groups

The clinical efficacy was different between the two groups at different time points, as shown in Table 2. The baseline and endpoint treatment variables were also compared in each group [Table 3]. These results indicated that at week 52, the monotherapy group had better liver function recovery compared with the add-on treatment group (ALT: 22.0 [17.0, 35.5] *vs.* 25.5 [18.8, 34.0] U/L, $P = 0.009$; aspartate aminotransferase [AST]: 27.0 [21.5, 31.0] *vs.* 30.0 [23.5, 37.0] U/L, $P = 0.019$), but there was no significant difference in ALT and AST levels between the two groups at week 104.

Table 1: Baseline clinical data of the treatment-naïve hepatitis B virus patients in the two-treatment groups.

Variables	NAs monotherapy ($n=56$)	Combination therapy ($n=73$)	Statistics	P
Age (years)	45.5 ± 12.7	42.9 ± 12.2	0.702*	0.489
Male, *n* (%)	35 (62.5)	48 (65.8)	0.146†	0.702
BMI (kg/m^2)	23.1 ± 3.1	23.4 ± 2.9	−0.761*	0.536
ALT (U/L)	59.0 (43.0, 100.5)	50.0 (30.8, 107.5)	−0.481‡	0.631
ALT >40U/L, *n* (%)	15 (26.8)	23 (31.5)	0.340†	0.560
AST (U/L)	45.0 (36.0, 76.5)	48.5 (34.0, 86.8)	−0.180‡	0.850
AST >40U/L, *n* (%)	14 (25.0)	21 (28.8)	0.227†	0.633
TBil (μmol/L)	14.5 ± 6.9	14.8 ± 8.8	−0.266*	0.791
AFP (ng/mL)	4.5 (2.8, 7.1)	5.7 (3.0, 18.8)	−1.318‡	0.187
Transient elastography value (kPa)	17.3 (10.1, 25.2)	14.3 (9.9, 18.9)	−1.262‡	0.207
PLT (×10^9/L)	127.3 ± 50.8	122.8 ± 53.1	0.946*	0.643
HBV DNA ($\log_{10}$ U/mL)	5.68 ± 1.23	5.56 ± 1.44	0.232*	0.636
HBsAg ($\log_{10}$ U/mL)	3.26 (2.82, 3.71)	3.30 (2.92, 3.95)	−0.211‡	0.873
HBeAg positive, *n* (%)	28 (50.0)	32 (43.8)	0.484†	0.487

Data are shown as the mean ± standard deviation (SD), *n* (%), or median (Q1, Q3). *t test; †χ^2 test; ‡Mann-Whitney U test. NAs: Nucleos(t)ide analogues; BMI: Body mass index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TBil: Total bilirubin; AFP: Alpha-fetoprotein; PLT: Platelet; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen.

Table 2: On-treatment laboratory examination comparative results at different time points between the two groups.

Variables		Baseline	26 weeks	P	52 weeks	P	78 weeks	P	104 Weeks	P
ALT (U/L)										
	Monotherapy	59.0 (43.0, 105.5)	26.0 (19.0, 37.5)	0.975	22.0 (17.0, 35.5)	0.009	21.0 (17.0, 32.0)	0.269	21.0 (15.0, 33.8)	0.583
	Add on	50.0 (30.8, 107.5)	28.0 (20.0, 36.0)		25.5 (18.8, 34.0)		26.0 (21.8, 31.8)		23.0 (17.3, 35.3)	
ALT >40 U/L										
	Monotherapy	15 (26.8)	8 (14.3)	0.738	6 (10.7)	0.469	1 (1.8)	0.228	4 (7.1)	0.948
	Add on	23 (31.5)	12 (16.4)		11 (15.1)		6 (8.2)		5 (6.8)	
AST (U/L)										
	Monotherapy	45.0 (36.0, 76.5)	31.0 (23.0, 37.4)	0.622	27.0 (21.5, 31.0)	0.019	25.0 (20.5, 29.0)	0.373	24.0 (19.5, 30.0)	0.697
	Add on	48.5 (34.0, 86.8)	28.5 (24.0, 34.8)		30.0 (23.5, 37.0)		26.0 (21.8, 31.8)		25.0 (20.0, 32.5)	
ALB (g/L)										
	Monotherapy	46.8 ± 5.5	45.3 ± 2.9	0.799	45.3 ± 2.1	0.208	46.5 ± 4.3	0.910	48.2 ± 4.7	0.774
	Add on	42.5 ± 4.9	45.4 ± 3.8		44.4 ± 4.3		45.1 ± 4.1		47.8 ± 6.3	
TBil (μmol/L)										
	Monotherapy	14.5 ± 6.9	14.3 ± 5.4	0.781	15.9 ± 7.0	0.125	14.3 ± 5.5	0.292	14.8 ± 6.3	0.086
	Add on	14.8 ± 8.8	14.0 ± 6.2		13.8 ± 7.0		13.2 ± 5.8		12.4 ± 5.0	
AFP (ng/mL)										
	Monotherapy	4.5 (2.8, 7.1)	3.3 (2.3, 4.1)	0.115	2.7 (2.0, 3.7)	0.015	2.2 (1.8, 2.9)	0.001	2.2 (1.8, 3.1)	0.022
	Add on	5.7 (3.0, 18.8)	3.6 (2.3, 5.6)		3.6 (2.3, 5.1)		3.1 (2.4, 4.6)		3.2 (2.0, 4.3)	
Transient elastography measurement										
	Monotherapy	17.3 (10.1, 25.2)	11.3 (7.5, 16.4)	0.579	10.5 (5.8, 15.4)	0.609	8.6 (5.6, 12.3)	0.792	7.8 (5.4, 11.1)	0.028
	Add on	14.3 (9.9,18.9)	10.1 (6.8,16.6)		9.0 (6.2, 13.6)		7.9 (5.8, 11.1)		6.6 (4.9, 9.8)	

Data are shown as the mean ± standard deviation (SD), *n* (%) or median (Q1, Q3). Monotherapy means that patients in this group only received ETV treatment. Add on means that patients in this group received add-on pegylated interferon alfa-2a to ongoing ETV treatment. There were few patients HBV DNA viral load >500 IU/mL at week 78 and 104, so the data was not comparable. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALB: Albumin; TBil: Total bilirubin.

Table 3: Clinical efficacy at baseline and the 2 years of the study in each group.

Variables	Monotherapy			Combination therapy		
	Baseline	2 years	*P*	Baseline	2 years	*P*
RBC ($\times 10^{12}$/L)	4.7 ± 0.6	4.3 ± 0.5	0.565	4.6 ± 0.6	4.9 ± 0.5	0.023
WBC ($\times 10^{9}$/L)	5.4 ± 2.1	5.2 ± 1.4	0.618	5.0 ± 0.5	5.2 ± 1.7	0.462
PLT ($\times 10^{9}$/L)	127.3 ± 50.8	150.8 ± 65.9	0.061	122.8 ± 53.1	166.9 ± 60.6	<0.001
ALT (U/L)	59.0 (43.0, 105.5)	21.0 (15.0, 33.8)	<0.001	50.0 (30.8, 107.5)	23.0 (17.3, 35.3)	0.007
AST (U/L)	45.0 (36.0, 76.5)	24.0 (19.5, 30.0)	<0.001	48.5 (34.0, 86.0)	25.0 (20.0, 32.5)	<0.001
ALB (g/L)	46.8 ± 5.5	48.2 ± 4.7	0.320	42.5 ± 4.9	47.8 ± 6.3	<0.001
TBil (μmol/L)	14.5 ± 6.9	14.8 ± 6.3	0.814	14.8 ± 8.8	12.4 ± 5.0	0.142
AFP (ng/mL)	4.5 (2.8, 7.1)	2.2 (1.8, 3.1)	<0.001	5.7 (3.0, 18.8)	3.2 (2.0, 4.3)	<0.001
Transient elastography value (kPa)	17.3 (10.1, 25.2)	7.8 (5.4, 11.1)	<0.001	14.3 (9.9, 18.9)	6.6 (4.9, 9.8)	<0.001
HBV viral load ($\log_{10}$ U/mL)	5.7 ± 1.2	<500	<0.05	5.6 ± 1.4	<500	<0.05
Liver histology						
Inflammation grades	2.0 (2.0, 3.0)	1.0 (1.0, 1.0)	<0.001	2.0 (2.0, 3.0)	1.0 (1.0, 1.3)	0.001
Fibrosis stage	3.0 (2.0, 4.0)	1.5 (1.0, 3.0)	0.021	3.0 (2.0, 4.0)	1.5 (1.0, 3.3)	0.011
Ishak fibrosis score	4.0 (1.0, 4.0)	2.0 (1.0, 3.8)	0.470	4.0 (3.0, 4.0)	1.5 (1.0, 3.2)	0.001

All data are presented as the mean ± standard deviation or median (Q_1, Q_3) as appropriate. RBC: Red blood cell; WBC: White blood cell; PLT: Platelet; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALB: Albumin; TBil: Total bilirubin; AFP: Alpha-fetoprotein.

Table 4: The number of patients who had HBsAg, HBeAg clearance, and/or seroconversion with emerging antibody during the research period.

Variables	0 week	26 weeks	52 weeks	78 weeks	104 weeks
HBsAg lost					
Monotherapy	0	0	1	1	1
Add on	0	0	0	1	3
HBsAb acquire					
Monotherapy	0	0	1	1	1
Add on	0	0	3	3	3
HBeAg lost					
Monotherapy	0	1	4	5	7
Add on	0	2	5	6	8
HBeAb acquire					
Monotherapy	0	1	4	4	6
Add on	0	1	4	5	7

Data are presented as the number of patients. HBsAg: Hepatitis B surface antigen; HBsAb: Hepatitis B surface antibody; HBeAg: Hepatitis B e antigen.

The results also showed that the monotherapy group might have favorable AFP decrease compared with the combination therapy group (2.2 [1.8, 3.1] *vs.* 3.2 [2.0, 4.3] ng/mL, $P = 0.022$). During the research, some patients have consistently high levels of AFP. But CT or MRI showed no evidence of abnormal nodules or liver cancer in these patients with high AFP. At the end of 2 years, the mean ALT and AST levels and the transient elastography value decreased significantly in each group compared with baseline data.

HBsAg/HBeAg clearance or seroconversion results

At week 26, patients in both groups only received ETV for treatment, and none of them showed HBsAg loss, whereas some patients began to lose and/or seroconvert HBeAg.

At week 52, one patient in the monotherapy group but none in the combination therapy group had lost HBsAg. In both groups, another three patients had lost and/or seroconverted HBeAg. The patient who had lost HBsAg in the monotherapy group also had an undetectable HBV viral load, but his liver function test results remained abnormal during the treatment. Additionally, before receiving ETV monotherapy treatment, the patient had already been followed-up in our department for 2 years with slightly high ALT and AST levels and HBV viral load.

At week 78, one patient from the combination group had lost and/or seroconverted HBsAg.

At the end of our study, there was altogether one patient who lost HBsAg in the monotherapy group while three patients in the combination therapy group showed HBsAg clearance (1.8% [1/56] *vs.* 4.1% [3/73], $P = 0.809$). There was also no significant difference in the HBeAg conversion rate (12.5% [7/56] *vs.* 11.0% [8/73], $P = 0.787$). All data are presented in Table 4. Additionally, Figure 2A and 2B

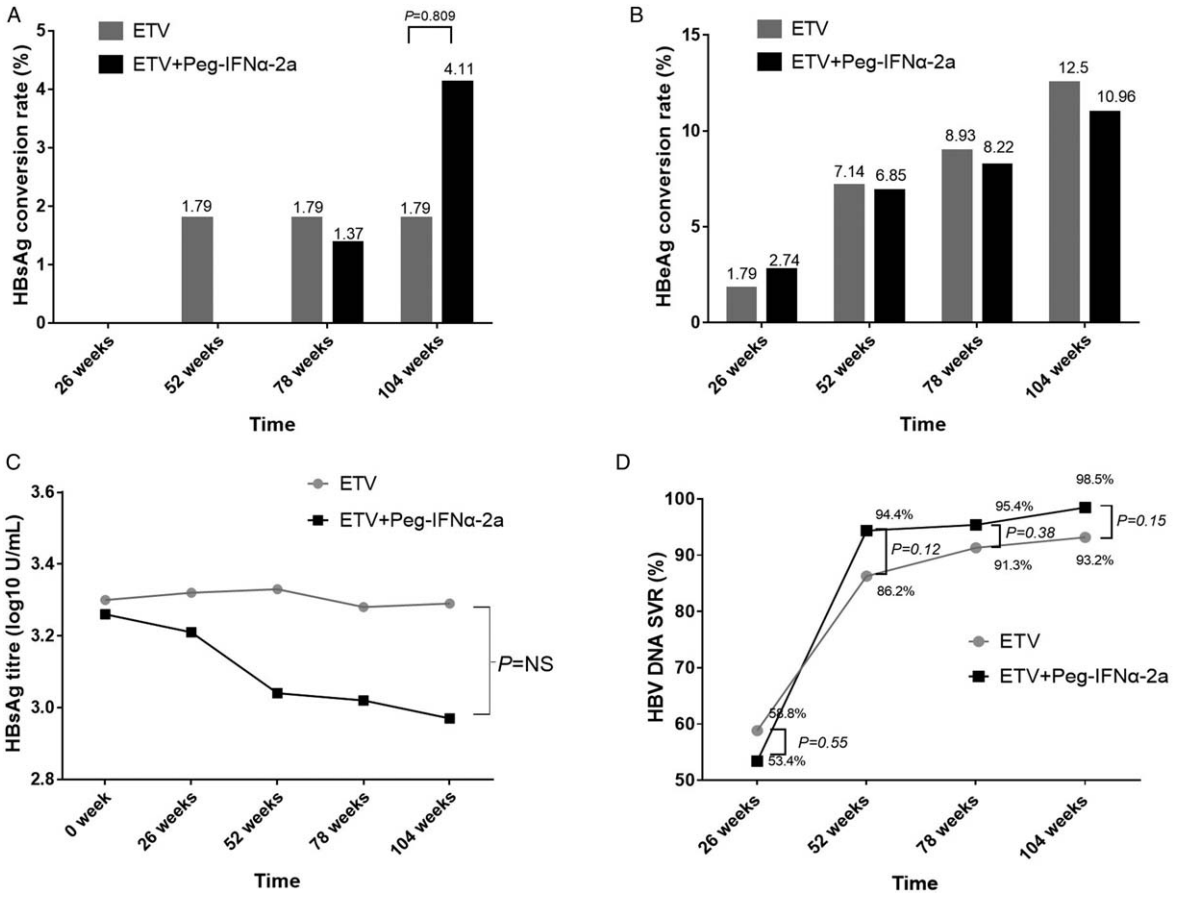

Figure 2: (A, B) The rate of patients who had HBsAg, HBeAg clearance and/or seroconversion with respective antibody formation. (C) The change in HBsAg levels during treatment in both groups. (D) Sustained suppression of HBV DNA at different time points. There was no significant difference in the HBsAg/HBeAg conversion rate, HBsAg levels and sustained suppression of HBV DNA between two groups at the end of the trial. But there is a tendency that combination therapy has a lower HBsAg level than ETV monotherapy. ETV: Entecavir; HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen; IFN: Interferon.

provide supplementary information about the total percentage of patients who lost HBsAg, HBeAg and/or seroconverted with the respective antibody formation at different time points. Figure 2C shows the tendency that HBsAg levels decease in two group. Figure 2D presents the results of sustained suppression of HBV DNA. There was no significant difference in sustained suppression of HBV DNA between the two groups.

Complementary liver histology results

Combination therapy showed better histology improvement than the monotherapy group (mean liver stiffness value 6.6 [4.9, 9.8] *vs*. 7.8 [5.4, 11.1] kPa, $P = 0.028$). The transient elastography value decreased significantly in each group compared with baseline data. Liver histology improved remarkably in both groups after 2 years. The results are shown in Figures 3 and 4. The Supplementary Table 1, http://links.lww.com/CM9/A235 showed patients' numbers of liver fibrosis stage.

Adverse events

During treatment, three patients showed thyroid dysfunction (one patient in monotherapy) and two patients had granulopenia (both in the combination therapy group). Six patients had a fever in the combination therapy group after they received their treatment. One patient felt fatigued in the ETV monotherapy group [Table 5].

Table 5: Incidence of discontinuation of treatment and adverse events (*n* [%]).

Variables	Monotherapy (*n* = 56)	Combination therapy (*n* = 73)
Discontinuation		
For safety reasons	4 (7)	5 (7)
For other reasons	8 (14)	3 (3)
Adverse events		
Thyroid dysfunction	1 (2)	0
Granulopenia	0	2 (3)
Fever	0	6 (8)
Fatigue	1 (2)	0

Discussion

In present, various therapies have proceeded for chronic hepatitis B, but the optimal regimen remains unclear. The clinical cure rate of combined treatment with NAs and peg-IFN is not sufficient to treat naïve chronic hepatitis B patients. Peg-IFN monotherapy was also found to be

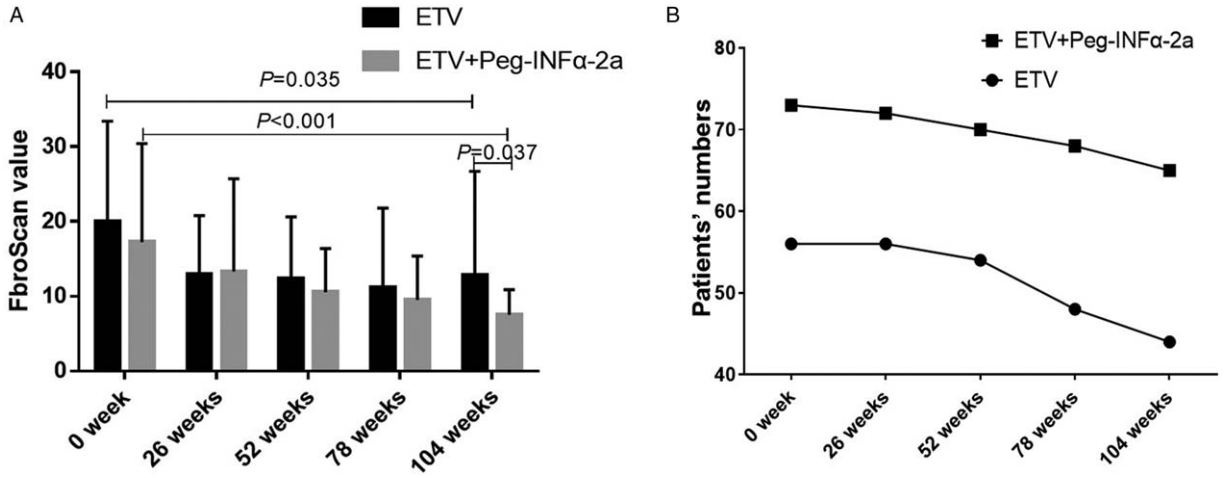

Figure 3: The changes in transient elastography value (A) and patients' numbers (B). ETV: Entecavir; IFN: Interferon.

efficient for HBsAg loss and seroconversion, but combination therapy was thought to cause more adverse events.[12,13] However, other studies have reported that the therapeutic efficacy of NAs combined with peg-IFN was better than monotherapy.[14] NAs can directly inhibit HBV DNA replication, while peg-IFNα-2a as an immunomodulator can enhance the innate and adaptive immune responses to play a synergistic anti-viral role.[15,16] In one study,[10] the addition of NAs to peg-IFNα-2a therapy enhanced the virologic response in chronic hepatitis B patients who did not have an early response to peg-IFNα-2a. This suggests that in patients with an early poor virologic response to Peg-IFNα-2a, the addition of NAs could inhibit viral replication. A trial directed by Ning *et al*[17] also found that patients who switched from ETV to peg-IFNα-2a significantly had increased rates of HBeAg seroconversion and HBsAg loss. But in our research, there was no significant difference in the HBsAg/HBeAg conversion rate. However, we found the tendency that HBsAg levels decease more quick in combination group. Presently, a new switching study[18] showed that HBeAg-positive chronic hepatitis B patients who switched from NAs to pegylated IFN achieved 12.5% and 16.2% HBeAg seroconversion and HBsAg loss, respectively. In a recent study, patients on long-term NA who are unlikely to meet therapeutic goals can achieve high rates of HBsAg loss by switching to Peg-IFN alfa-2a.[19] It seems add-on therapy resulted in a more viral decline and appeared to prevent relapse after stopping ETV compared with monotherapy. Therefore, based on this synergistic mechanism, combination therapy may be an ideal method for chronic HBV patients. However, we did not observe these results. In our study, we did not find a significant difference in clinical efficacy between the two groups, which is similar to other studies that reported that combination therapy failed to improve clinical efficacy.[20] However, a recent meta-analysis also showed that combination therapy increased the virologic response and sustained virological response.[7] And during the 2-years following-up, we found that patients had relatively higher levels of ALT, but lower ALB in our study. The reason maybe is that the sample size is small and some patients who have sustained liver damage during the treatment influence the mean level of ALT and ALB. Whereas, adding peg-IFNα-2a to adefovir dipivoxil or ETV showed better clinical efficacy in HBeAg-negative patients.[21]

In this randomized controlled trial, our results showed that combination therapy did not have better anti-viral efficacy than ETV monotherapy for sustained virologic suppression, HBsAg/HBeAg clearance, and seroconversion, but its histologic results showed improvement in the combination group. Additionally, the rate of HBsAg/HBeAg clearance and seroconversion was not different from combination therapy compared with ETV monotherapy after adding peg-IFNα-2a. But a recent retrospective cohort study[22] showed that patients in the peg-IFN add-on therapy group were more likely to achieve HBeAg seroconversion (44% *vs*. 6%; $P < 0.0001$) compared with those in the ETV monotherapy group. The reason may be that the duration of the added peg-IFNα-2a administration was too short, and the difference was not significant. Although some guidelines recommend 48 weeks of peg-IFN administration for patients with chronic hepatitis B, we administered 26 weeks of peg-IFN add-on therapy because of the side effects and cost of the drug. Additionally, Brouwer *et al*[23] indicated that peg-IFN add-on therapy led to a higher proportion of HBeAg response compared with ETV monotherapy for HBeAg-positive chronic hepatitis B. The inclusion and exclusion criteria for patients may lead to differences in the outcomes.

Generally speaking, the sustained benefit of combination therapy requires further investigation. In our 2-year study, we compared the sustained efficacy between the two groups at different time points and we found that there was no significant difference between sustained suppression of HBV DNA in the two groups. However, this was not inconsistent with the results of a recent study, which showed that combination therapy enhanced the virologic response and sustained suppression of HBV DNA.[7] The ideal result of anti-HBV therapy is HBsAg seroconversion, which often means a viral clearance. Achieving full viral clearance remains a challenge. During the trial, three

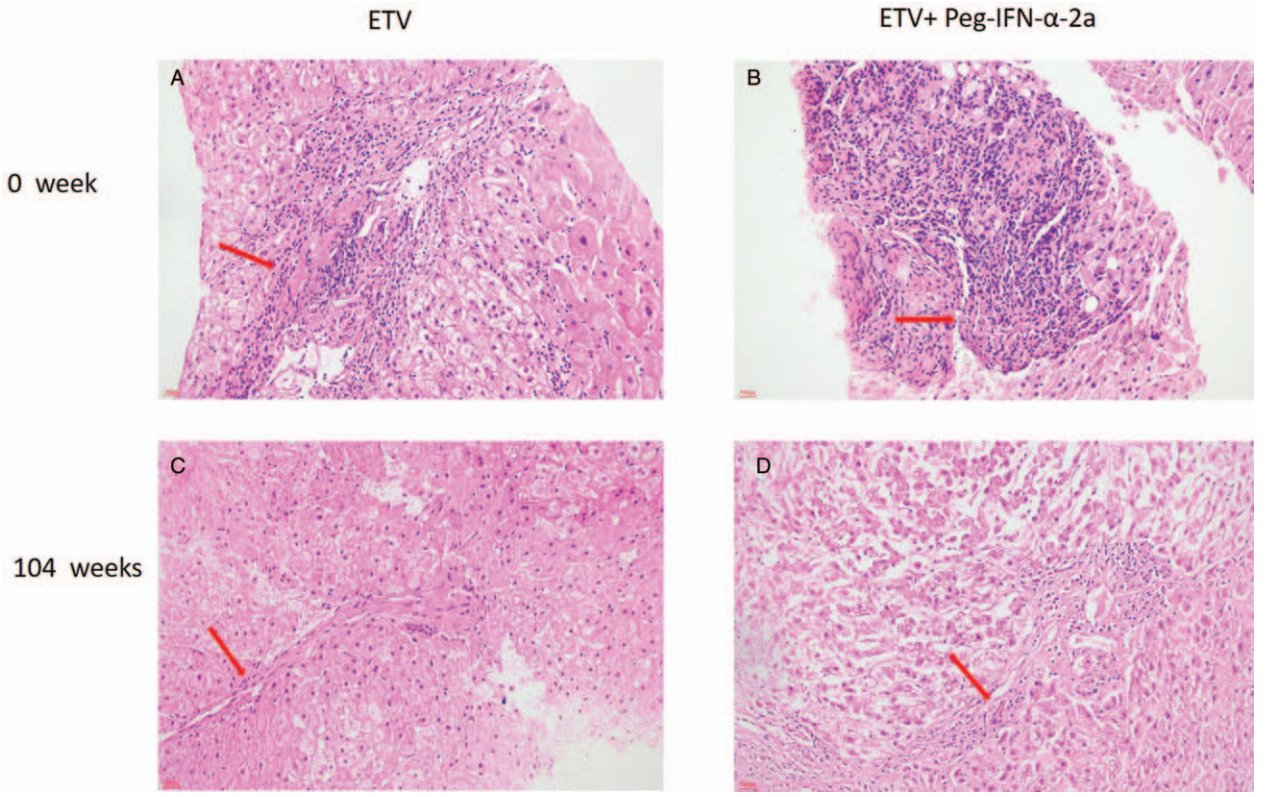

Figure 4: Liver biopsy examination results (hematoxylin-eosin staining, original magnification ×200). (A) Histological examination results of the liver indicated inflammation grade 1 and fibrosis stage 2 in an ETV monotherapy group patient before treatment. (B) Histologic examination results of the liver indicated inflammation grade 2 and fibrosis stage 2, in an ETV+ peg-IFN-α-2a group patient before treatment. (C) Second liver biopsy in the same patient in the ETV monotherapy group after 2 years of treatment. The results indicate inflammation grade 1 and fibrosis stage 1. (D) Second liver biopsy of the same patient in the ETV+ peg-IFN-α-2a group after 2 years of treatment. The results indicate inflammation grade 1 and fibrosis stage 1. The arrows point to the inflammation or fibrosis area. ETV: Entecavir; IFN: Interferon.

patients (two in the monotherapy group and one in the combination group) had hepatitis relapses even though their HBsAg was cleared and their viral load was undetectable during treatment. This phenomenon requires further study.

For histology improvement, we analyzed the transient elastography value and histological fibrosis score between the first day of treatment and the end of therapy. A significant difference in the transient elastography value was seen between the monotherapy and combination groups. The transient elastography value of combination groups decreased more than the monotherapy group. However, the ALT and AST recovery results were not in agreement with the transient elastography value. The monotherapy group tended to have lower ALT, AST and lower AFP levels compared with combination therapy, but not the transient elastography results. Liver histology improved remarkably in each group after treatment for 2 years. Some studies showed that a combination of peg-IFNα-2a may improve liver histology better than monotherapy by immunologically modulating activity of effector T-cells, which generate a robust cytotoxic T lymphocyte (CTL) response.[24] CTLs can kill infected cells, so it causes an increase in transaminase levels. Some studies believe that under treatment with PEG-IFN-alfa-2a, CTLs can induce cell apoptosis by releasing perforin and cause temporary liver function damage.[25,26] And there is a research that CD+T cell activation is related to liver inflammation and the pathogenesis of hepatitis.[27] Additionally, it improved histology following long-term treatment. We also found that the AFP levels in the monotherapy group were lower than those in the combination group during the treatment. Lower post-treatment AFP levels were reported to be significantly correlated with liver fibrosis regression.[28] This phenomenon may be explained by the original baseline AFP levels in the combination therapy that were higher than those in the monotherapy group, although there is no significant difference between the two groups. Liver biopsy histological scores were evaluated at the end of the study, and the combination therapy was likely to have improved histology results compared with monotherapy, but the difference was not significant.

The most frequent adverse events reported in the combination group were fatigue, headache, fever, and myalgia.[29-31] These adverse events were mostly related to adding Peg-IFNα-2a. As expected, more chronic hepatitis B patients taking combination therapy had side effects compared with those taking monotherapy in our study. Three patients had thyroid dysfunction, two patients had granulopenia, and one patient had fatigue in the ETV monotherapy. Therefore, attention must be paid to the side effects of combination treatment.

There are some limitations in this trial, such as the small sample size, especially because some patients were lost to follow-up during treatment resulting from pregnancy,

economic conditions, or the patient was unwilling to continue in the trial. However, the advantage of this study was that it was a randomized controlled prospective study. We evaluated the changes in ALT, AST, HBsAg/HBeAg clearance and seroconversion rate, and sustained virologic response at regular intervals, and we observed liver histological examination results. We also studied the effect of reducing the use time of Peg-IFNα-2a.

In conclusion, for treatment-naïve patients with chronic hepatitis B, both monotherapy and combination therapy successfully improved liver function and histology. However, combination therapy did not show a better effect on HBsAg and HBeAg clearance and seroconversion or satisfactorily reduce HBV-DNA to an undetectable level. Our data also showed that combination therapy played a more positive role in reversing hepatic fibrosis than did monotherapy, but the safety of combination therapy requires further study. The curative effect of combination therapy and monotherapy also requires further study.

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Conflicts of interest

None.

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