Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

The effect of thymopentin add-on in hepatitis B e antigen positive chronic hepatitis B after virus suppression by peginterferon plus entecavir therapy

Yanpin Ma[1], Xuli Bao[1], Fang Xiong[2], Jinhuan Wang[3], Na Gu[1], Jia Guo[1], Huili Wu[3], Jun Lu[1,*]

[1] Hepatology and Cancer Biotherapy Ward, Beijing You-An Hospital, Capital Medical University, Beijing, China
[2] Hepatology and Endocrine Ward, Beijing You-An Hospital, Capital Medical University, Beijing, China
[3] International Medical Department, Beijing You-an Hospital, Capital Medical University, Beijing, China

Correspondence to: lujun98@ccmu.edu.cn

Received December 18, 2018; **Accepted** February 20, 2019; **Published** February 28, 2019

Doi: http://dx.doi.org/10.14715/cmb/2019.65.2.12

Abstract: Hepatitis B virus (HBV) covalently closed circular DNA (cccDNA) was positively correlated with serological hepatitis B surface antigen (HBsAg) levels in hepatitis B e antigen (HBeAg) positive chronic hepatitis B (CHB) patients. We evaluated whether Thymopentin (TP5) and interferon (IFN-a) had a synergic effect on HBV cccDNA and the effect of TP5 addition therapy on HBsAg clearance in CHB patients. Real-time PCR experiments were performed to test cccDNA in HepG2.2.15 cells. 45 HBeAg-positive CHB patients had been distributed into two groups randomly. Treatment group: 23 patients were treated with a 24-week TP5 on the basis of the treatment entecavir (ETV) and peginterferon alfa-2a (PegIFN alpha-2a). Control group: 22 patients were treated with ETV and PegIFNa-2a. The study period was 72 weeks. In HepG2.2.15 cells, TP5 5ug/ml and 10ug/ml respectively combined with IFN-a 2ku/ml could potently inhibit cccDNA level at 72 hours ($P<0.05$). In clinical study, mean HBsAg levels in two groups are not significantly different at different time points ($p=0.112$). However, changes of mean HBsAg levels in TP5 add-on group at different time points are significantly different ($p<0.05$). Patients with HBsAg levels <1500IU/ml in control group had higher HBsAg levels compared with patients with HBsAg levels <1500IU/ml in TP5 add-on group ($P=0.019$). The latter had the most pronounced HBsAg reduction. TP5 and IFN had a synergic effect on inhibiting cccDNA levels in HepG2.2.15 cells; Patients in treatment group showed no extra side effects compared with the control group. 24 weeks TP5 add-on treatment was safe and had a tendency to accelerate the decline of HBsAg when HBV-DNA was undetectable.

Key words: Hepatitis B surface antigen; Covalently closed circular DNA; Thymosin alpha 1; Thymopentin; HepG2.2.15 (RRID: CVCL_L855) hepatoma cells.

Introduction

Nowadays Liver disease caused by Hepatitis B virus (HBV) infection has jeopardized the health of more than 350 million people worldwide. (1) Though many endeavors have been done on the prevention and therapy of HBV infection, it still remains an unresolved problem.

In chronic hepatitis B (CHB) infection, hepatitis B surface antigen (HBsAg) loss is regarded as the optimal treatment endpoint by Clinical Practice Guidelines. (2) HBsAg loss does not mean the eradication of HBV, because the current antiviral treatment cannot eliminate covalently closed circular DNA (cccDNA) and integrated DNA. The former is intracellular HBV replication intermediate existing in the nucleus of infected cells. (3) The latter is HBV integrated into host DNA which is contributing to carcinogenesis. Both of them are the sources of HBsAg. (4-6) Therefore, the eradication of HBV is rarely obtained.

Nucleos(t)ide analogues (NA) and peginterferon (Peg-IFN) are hitherto two standard therapies of care for CHB patients. NAs therapy does not target the cccDNA which is unable to eliminate the infected hepatocytes, HBV viremia occurs in most patients with cessation of NA therapy. (7) IFNa combines direct antiviral effect and immunomodulation together. It can target cccDNA transcription and contribute to the decline of HBsAg. However, its effect is only limited in a minority of individuals. (8)

In CHB, the antiviral immune responses are defective due to virus-host interaction, including impaired noncytolytic antiviral capacity of natural killer (NK) cell, (9) the activation of regulatory T cells and the defective functionality of HBV-specific CD8 T cells. (7, 10) Studies showed that when the virus replication was inhibited by NAs and the immune function of IFN-a could be enhanced. The potent antiviral therapy combined with immunomodulator therapy could interplay with each other and lead to a better outcomes for CHB treatment. (11-13) This indicates the function of immunomodulators cannot be neglected. Thymosin alpha 1 (Tα1) is an immunomodulator. E. Loggi et al found that Ta1 combined IFN-a together could increase IL-2 production and reverse the IFN-induced increase of IL-10, which is beneficial for the treatment of CHB. (14) Ta1 can also regulate the immune system by enhancing innate and adaptive immunity interaction. (15, 16) Therefore, it is necessary to further study the synergic effect of potent antiviral therapy combined immunomodulators therapy.

Thymopentin (Arg–Lys–Asp–Val–Tyr, TP5) is a synthetic pentapeptide of 49 amino acids which contains thymic hormone and shows immunomodulator activity. (17) Same as Tα1, characterized by immunomodulatory

activities, (18) TP5 has been used in HBV treatment for many years.

The aim of our study was to evaluate whether TP5 and IFN-a had a synergic effect on cccDNA and the effect of TP5 addition therapy on HBsAg in CHB patients. In vitro study, we investigated the effect of TP5 and IFN-a on nuclear hepatitis B virus cccDNA in HepG2.2.15 cells. In clinical study, we assessed the effect of TP5 add-on in HBeAg positive patients after HBV suppression by PegIFN alpha-2a and entecavir (ETV) combination therapy.

Materials and Methods

Cell cultures and drug treatments

HepG2.2.15 (RRID: CVCL_L855) hepatoma cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. IFN-α was used at a final concentration from 0.5 to 5 KU/ml and was added directly to the culture medium. (19, 20) TP5 was used at a final concentration from 10-100 ug/ml and was added directly to the culture medium.

HBV cccDNA quantification

HepG2.2.15 cells were collected at the indicated times, and DNA was isolated using DNeasy Blood and Tissue Kit (Qiagen Inc., Germany, Cat No./ID : 69504) according to the manufacturer's instructions. The DNA was eluted in 50ul of nuclease-free water. 500 ng aliquots of each extracted DNA were treated for 45 minutes at 37°C with 10 U plasmid safe DNase I (Epicentre Inc., Madison, WI). DNase was inactivated by incubating the reactions for 30 minutes at 70°C. Real-time PCR experiments were performed in a CFX 96 (BioRad Laboratories. California, USA) using a 20ul reaction volume containing 20 ng of DNA, 2*Taqman mix (Takara Bio Inc, Tokyo, Japan), 0.2 mmol/l probe and primers. Forward and reverse primers were as follows: 5'-GGGGCGCACCTCTCTTTA-3' and 5'-AGGCACAGCTTGGAGGC-3', respectively. Hybridization probes were 5'-FAM-TCACCTCTGCCTAATCATCTC-TAMRA-3'. Amplification was performed as follows: 95°C for 10 minutes, then 45 cycles of 95°C for 10 seconds 58°C for 10 seconds. Serial dilutions of a plasmid containing a monomeric genotype d HBV insert (pHBV1.3.) were used as quantification standards. (20) Globin is amplified as reference.

Patients and methods

45 patients were enrolled in current clinical study. They came from 90 patients with hepatitis B antigen (HBeAg) positive CHB who participated in a previous study conducted in our department. 90 patients who met the following entry criteria were eligible for inclusion: 18–65 years old with serum HBsAg-positive for at least six months; serum alanine aminotransferase (ALT) with a value of 2-10×times upper limit of normal (ULN) at least two occasions, more than one month apart; serum HBeAg and HBV-DNA had been documented on two occasions, more than one month apart, within a period of four months before randomization. Subjects were required to have compensated liver disease without absence of clinical evidence of esophageal varices, ascites, hepatic encephalopathy, and imaging features suggestive of cirrhosis on ultrasonography. The exclusion criteria included: other antiviral, immunomodulatory agents or corticosteroid treatment within six months before entry; co-infection with human immunodeficiency virus, hepatitis C or hepatitis D virus; a history of malignancy, intravenous drug abuse, or evidence of other forms of liver disease. Patients with other significant medical or psychiatric problems were also excluded. (21, 22) The enrolled patients were given 180 mg of PegIFN alpha-2a (Pegasys; Roche) once weekly+ 0.5mg of entavir (ETV) once daily for 48 weeks. After then, 45 patients with HBeAg positive and HBV-DNA<100IU/ml were studied. They were distributed into two groups randomly (1:1). Patients in control group (n=22) continued PegIFN alpha-2a combined ETV therapy for another 72 weeks. Patients in treatment group (n=23) were given 180 mg PegIFN alpha-2a once weekly, 0.5mg ETV once daily and 10mg subcutaneous TP5 (Thymopentin for injection; Beijing SL Pharmaceutical co., LTD) three times a week for 24 weeks. After then, PEG-IFN a-2a and ETV treatment were given to the patients till 72 week. The study was approved by the Ethics Committee on Clinical Investigation of the Beijing You'an Hospital, Capital Medical University (Beijing, China). Written informed consent was obtained from all participating individuals before enrollment into the study. The trial was carried out in accordance with the Helsinki declaration on experimentation on human subjects.

Laboratory assays

During each visit, patients were examined and blood samples were taken for biochemical and hematological analyses, and for evaluation of HBsAg, HBeAg and HBV-DNA. After the first clinic visit at baseline (week 0), patients were evaluated at the end of week 12, week 24, week 48 and week 72. All patients were followed up till 72 weeks. HBV-DNA was quantified by fluorescence quantitative polymerase chain reaction with lower limits of detection of 20 IU/mL. HBsAg and anti-HBs antibody were quantified by HBsAg quantitative Elecsys (Roche Diagnostics GmbH, Germany) with lower limits of detection of 0.05 IU/mL for HBsAg. Anti-HBs antibody level >10 IU/L was defined as positivity. The patient has a regular outpatient visit and blood tests, so the specimen were not stored and analyzed right away. Each specimen was tested 3 times and three parallel controls were set up at the time of detection.

Endpoints

The primary endpoint was safety, HBeAg seroconversion, HBsAg loss at the end of treatment. Secondary endpoint included HBV-NDA undetectable (<20IU/ml), and HBsAg kinetics during 72-week period.

Statistical analysis

HBsAg levels were logarithmically transformed for analysis. Categorical variables were compared by the chi-square test. Distributions of continuous variables were analyzed using the independent student's t test or the Mann-Whitney U test. Repetitive measure analysis of variance was used to analyze the difference of serum HBsAg titers at different time points in groups. Paired comparisons were used between baseline and 12 week, 24 week and 72 week in each group respectively

Table 1. Baseline characteristics of entrolled patients.

	Treatment Group	Control Group	p value
Male, *n* (%)	13 (56.5)	16 (72.7)	0.353
Age, year	31.23±7.843	32.06±9.477	0.764
Weight,kg	69.43±14.73	74.82±11.34	0.178
ALT, IU/l	35.28±18.46	30.56±20.94	0.571
TB	12.08± 3.81	12.37±3.89	0.702
ALB	45.70±2.44	41.65±8.33	0.113
HBV-DNA, n (%)			
< 20IU/ml	11 (47.8)	12 (54.5)	0.768
20-100IU/ml	12 (52.2)	10 (45.5)	0.768
HBsAg, log_{10}IU/ml	3.10±1.04	3.37±0.69	0.310
HBeAg, log_{10}IU/ml	1.25±0.93	1.48±0.76	0.407

Data are expressed as the mean ± SD or percentages. ALT, alanine aminotransferase; TB, total bilirubin; ALB, albuminate; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen.

whenever appropriate. The changes of HBsAg level in two groups were assessed by linear regression analysis. P<0.05 was considered statistically significant. Statistical analysis was conducted with SPSS version 20 (SPSS Inc., USA).

Results

To investigate the inhibition effect of IFN-a on established HBV cccDNA, HepG2.2.15 cells cells were treated with different concentration from 100-1000 IU/ml and analysis the cccDNA level at different time point. Within 24 hours, IFN-a had the strongest inhibitory effect on cccDNA, and the inhibitory effect was enhanced with the increase of concentration, the difference was significant when the IFN concentration was 2KU/ML and 5KU/ML (p<0.05). At 48 hours and 72 hours, there was no significant difference at different time points and concentrations (Figure 1A). After TP5 treatment, the level of cccDNA decreased significantly compared with the control. At the concentration of 5ug/ml, the relative level of cccDNA did not decline. The relative level of cccDNA decreased rapidly, when it increased to 10ug/ml. At 20ug /ml, the relative level of cccDNA decreased more rapidly, the difference was significant (P<0.05). CccDNA relative level decreased continuously as treatment was prolonged to 48 hour, but were not as rapidly as that at 24 hour. The difference at 10ug/ml and 20ug/ml was still obvious compared with the control (P<0.05) (Figure 1B). At 72 hour, cccDNA relative level stayed stable except that at 20ug/ml and the difference was significant at 20ug/ml compared with the control.

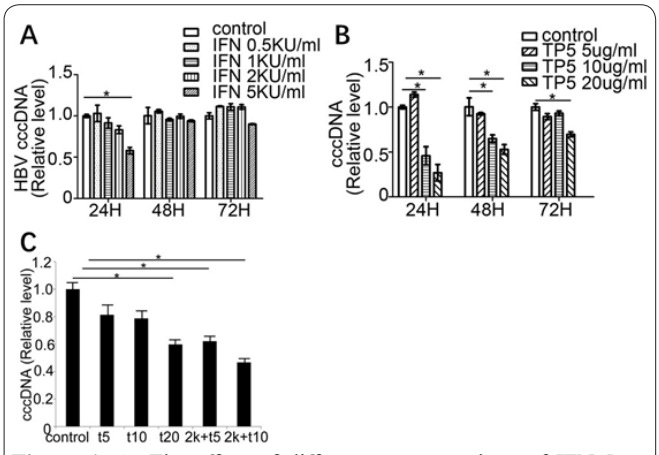

Figure 1. A: The effect of different concentrations of IFN-5 on cccDNA of HepG2.2.15 cells at different times; B: The effect of different concentrations of TP5 on cccDNA of HepG2.2.15 cells at different times; C: The effect of T5 with different concentrations and IFN combined with T5 on cccDNA in HepG2.2.15 at 72h. *P < 0.05. Each specimen was tested 3 times and three parallel controls were set up at the time of detection. The value of bar columns and error bars are Mean ± Standard Deviation.

The above results showed that when the concentration of IFN-a was 2ku/ml at 72 hours, it had no inhibition effect on cccDNA compared to the control. Therefore, we examined the effect of TP5 5ug/ml and 10ug/ml combined with IFN-a 2ku/ml respectively on cccDNA level at 72 hours to see whether the inhibition effect of IFN and TP5 can be enhanced. It showed that this combination could strongly inhibit cccDNA level, and the differences were significant compared to the control (p<0.05) (Figure 1C).

Baseline characteristics

From July 2013 to January 2016, 45 patients were studied. They were studied for another 72 weeks (Figure 2). Data on 45 patients were similar after the previous 48 weeks treatment with respect to sex, age, TB levels, ALT levels, ALB levels, serum HBV-DNA levels, HBsAg and HBeAg levels (Table 1). One patient in control group lost follow-up at week 24. One patient in treatment group lost follow-up at week 48. One patient in each group stopped therapy without medication.

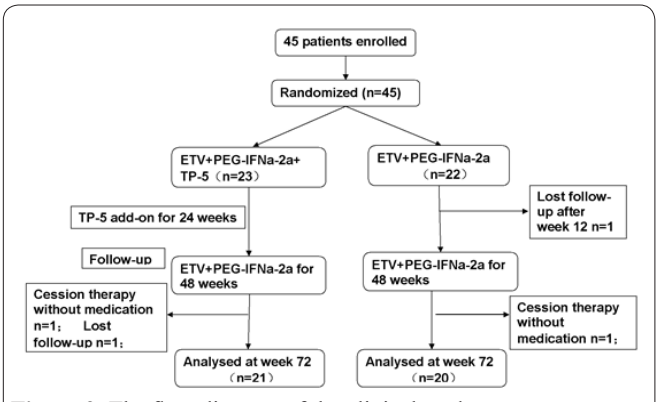

Figure 2. The flow diagram of the clinical study.

Table 2. Comparison of HBeAg seroconversion in two groups at week 12,24,48 and 72 .

	12w	24w	48w	72w
PEG-IFNa-2a+ETV+TP5	8.7% (2/23)	13.0% (3/23)	13.0% (3/23)	19.0% (4/21)
PEG-IFNa-2a+ETV	4.5% (1/22)	4.8% (1/21)	9.5% (2/21)	20.0% (4/20)
p value	1.000	0.609	1.000	1.000

PEG-IFNa-2a, pegylated interferon alfla-2a; ETV, entecavir; TP5, thymopentin.

Efficacy

At the end of the TP5 therapy (week 24), HBeAg seroconversion rates in two groups were shown in Table 2. HBeAg seroconversion rates at different time points had no differences in the two groups.

During the therapy and follow-up period, only one patient showed HBsAg loss at week 72 in the treatment group. There is no one showing HBsAg loss in the control group.

Serum HBsAg levels were compared between two groups by using repetitive measure analysis of variance. HBsAg levels in two groups were not significantly different (p=0.112). However, HBsAg levels at different time points decreased quickly in TP5 add-on group. The slopes of mean HBsAg level in TP5 add-on group and control group was -0.0045 and -0.0011 respectively (Figure 3A). Mean HBsAg decline at different time points were significantly different (p=0.008). Mean HBsAg decline compared to baseline level was 0.1107logIU/ml and 0.3041 logIU/ml respectively at week 24 and week 72 in TP5 add-on group, while it was 0.0036logIU/ml and 0.0764 logIU/ml respectively in PegIFN alpha-2a combined ETV group (Figure 3B). Compared to baseline HBsAg level, the decline were obvious in TP5 add-on group at week 24, week 48 and week 72 (p=0.015, 0.010 and 0.020 respectively). In contrary, PegIFN alpha-2a combined ETV group, HBsAg decline at 12,24,48 and 72 week compared to baseline level had no significant difference (P=0.251,0.926,0.215 and 0.367 respectively) (Figure 3C).

HBsAg changes among patients with baseline HBsAg levels <1500IU/ml and baseline HBsAg levels >1500IU/ml in two groups during the 72 weeks were depicted in Figure 3D. HBsAg levels in different groups were significantly different (P=0.000). Patients with HBsAg levels <1500IU/ml in control group had higher HBsAg levels compared with patients with HBsAg levels <1500IU/ml in TP5 add-on group (P=0.019).

Changes of HBsAg levels at different time points were showed in Figure 3E. Patients with baseline HBsAg levels > 1500IU/ml in TP5 add-on group (treatment group) had the most pronounced HBsAg reduction. Compared with baseline, Mean HBsAg levels declined by 0.2035logIU/ml and 0.5129 logIU/ml respectively at week 24 and week 72.

During both the study durations, viral loads were maintained undetectable (<100IU/ml). When using more sensitive method fluorescence quantitative polymerase chain reaction with lower limits of detection of 20 IU/ml, we found that HBV-DNA level of patients was between 20-100IU/ml. Then we compared the ratio of HBV-DNA <20IU/ml in each group at different time points between two group which was shown in Table 3. Two patients who stopped therapy without medication showed HBV relapse in the subsequent follow-up.

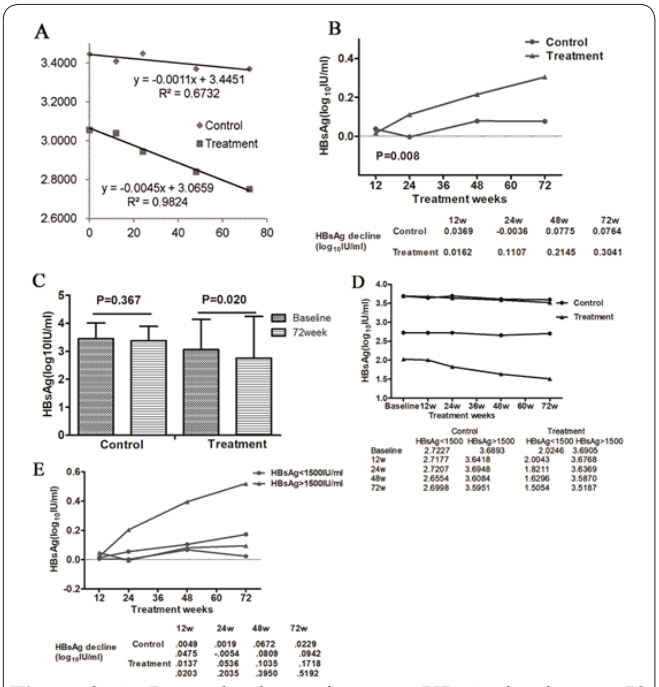

Figure 3. A: Dynamic change in serum HBsAg levels over 72 weeks in two groups; B: Mean serum HBsAg decline from baseline at different time points in two groups; C: Comparison the difference of serum HBsAg levels at baseline and 72 week in two groups; D: HBsAg changes among patients with baseline HBsAg levels <1500IU/ml and baseline HBsAg levels >1500IU/ml in two groups during the 72 weeks; E: Changes of HBsAg levels at different time points in different groups. Each specimen was tested 3 times and three parallel controls were set up at the time of detection.

Safety

Typical adverse effects including fatigue, reduced appetite, weight loss, a decreased platelet, neutrophil and white blood cell counts are comparable in two groups. These effects are known to occur with PegIFN alpha-2a therapy. None of the 45 patients required a dosage adjustment or cession of drugs due to the adverse effects. All of them were tolerant quite well. Consequently, TP5 add-on showed no evidence of extra side effect.

Table 3. Comparison of HBV-DNA < 20 in two groups at week 0,12,24,48 and 72.

	0w	12w	24w	48w	72w
PEG-IFNa-2a+ETV+TP5	47.8% (11/23)	52.2% (12/23)	60.9% (14/23)	78.3% (18/23)	81.0% (17/21)
PEG-IFNa-2a+ETV	31.8% (7/22)	40.9% (9/22)	57.1% (12/21)	61.9% (13/21)	80% (16/20)
p value	0.365	0.554	1.000	0.325	1.000

PEG-IFNa-2a, pegylated interferon alfla-2a; ETV, entecavir; TP5, thymopentin.

Discussion

In vitro study, we found both TP5 and IFN-a could inhibit nuclear HBV cccDNA in HepG2.2.15 cells. Especially, in the cells line experiment, it showed that at 72 hour IFN-a combined with low concentration of TP5 could greatly inhibited cccDNA. It indicated that IFN-a and TP5 together have a synergic effect on cccDNA at a relative low concentration. The effect of IFN-a on cccDNA in HepG2 hepatoma cells had been shown in previous studies. (19, 20) Most importantly, no extra adverse effect was found. Therefore, TP5 add-on was safe.

HBV cccDNA was positively correlated with serological HBsAg levels in HBeAg-positive CHB patients. (23) In clinical study, we found TP5 add-on had a tendency to accelerate HBsAg decline. Most importantly, no extra adverse effect was found. Therefore, TP5 add-on was safe.

In current clinical study, HBeAg seroconversion rate in treatment group and control group were comparable at 48 week (13.6% vs 12.8%, P=1.000). However, in the previous studies PegIFN alpha-2a monotherapy or PegIFN alpha-2a combined NAs led to a higher HBeAg seroconversion rate in CHB patients, which was above 20%. (24, 25) This is largely because our patients were "difficult-to-treat" patients. These 40 patients were called "difficult-to-treat" patients according to the study of Ning q et al who indicated that patients who failed to acquire HBeAg seroconversion but achieved HBV DNA suppression after a potent NA therapy for more than one year are difficult to treat. These patients possibly need a lifetime treatment possibly need a lifetime treatment. (26) Therefore, it is difficult for them to acquire HBeAg seroconversion. The existence of HBeAg means that CHB patients have not acquired immune control over chronic HBV infection. HBeAg seroconversion accelerates the probability of HBsAg loss and seroconversion, which has been shown to be an improved prognosis. (27) Therefore, enhancing the immune function by TP5 add-on is essential.

Lower HBsAg levels also mean higher chance of HBsAg seroclearance. (28) Meanwhile, HBsAg are modulated by the interaction between virus and host immune response. Patients with lower HBsAg levels mean a better immune control of HBV. (29, 30) In current study, 24-week TP5 add-on has a tendency to accelerate HBsAg decline. Specially, patients with baseline HBsAg levels<1500IU/ml in TP5 add-on group had the most striking HBsAg reduction. Mean HBsAg levels decreased by 0.3157logIU/ml after one year of TP-5 add-on treatment. A 10-year of NA Therapy study showed that serum HBsAg levels decreased 0.1 logIU/mL/year during decade-long NA therapy. (31) Furthermore, it should be noted that patients enrolled in our study had already been treated by ETV and PegIFN for one year. Studies indicated that HBsAg levels decreased quickly in the first year after antiviral treatment for the HBeAg-positive CHB patients. (32) Since HBsAg loss is less frequently achieved by current treatment agents, our finding is beneficial for the cure of CHB in some degree.

The existence of HBV cccDNA contributes to HBV persistence, host immune status also play important roles in HBV persistence. (10, 33) The prolonged exposure to HBV viremia and antigen load results in impairment or exhaustion of T cell function. The level of HBV replication and antigen load is related to the degree of them. (34, 35) Thus, by inhibiting HBV replication and reducing antigen level, it may facilitate T cell function reconstruction and prompt immunoregulator agent more effective. (10, 36) Meanwhile, NAs therapy could partly restored HBV-specific T cell responses by inhibition of HBV replication in CHB patients. (37-40) PegIFN alpha-2a could mediate NK cell functions, (41) but prompt T cell apoptosis and inhibit T cell proliferation (42). Ta-1 (thymosin alpha-1) could promote T cell maturation and differentiation (3) and enhance the antiviral activity of NK cells and macrophages cells (43). Thus, Ta-1 could coordinate the activity of the innate and adaptive T cell immunity (15). To date, Ta-1 was considered as an adjuvant treatment to maintain immune response for HBV infection due to its controversial clinical therapeutic effect. (17, 44, 45) However, its pleiotropic mechanisms on affected immune cells and the good therapeutic effect in tumor, immune deficiency and age et al had been proven in preclinical and clinical studies. (15) Therefore, we assumed that the addition of Ta-1 could play a synergic effect to enhance immune response when patients still suffered impaired immune function. The synergic effect of IFN and TP5 on cccDNA had been confirmed in our cells line study.

To date, there is no study to evaluate the efficacy of TP5, PegIFN alpha-2a and ETV in CHB patients. We conducted a randomized study for the first time. However, there are some limits about the study. First, it was a single-center study and the sample size can be enlarged. The further investigations should be conducted on a multicenter and a large number. Second, prolonged follow-up of patients would be better observing HBeAg seroconversion and HBsAg loss rate. Third, the particular mechanism of the function of TP5 and IFN-a was not illuminated in the cell line study, so more studies are needed to identify the specific mechanism of them. Finally, patients who were HBeAg positive and HBV-DNA>100IU/ml after 48 weeks ETV plus PegIFN alpha-2a therapy had not been enrolled due to change treatment regime.

In conclusion, 24 weeks TP5 treatment combined with ETV plus PegIFN alpha-2a was safe. TP5 add-on when HBV-DNA was undetectable had a tendency to accelerate the decline of HBsAg. Specially, the decline is obvious in patients with relative lower HBsAg levels, which would possibly improve rates of HBsAg loss. This therapeutic strategy exerts dual mechanism, including antiviral activity against HBV and immune modulation which will be helpful to the cure of CHB. It provides a new therapeutic concept and merits a further research.

The name of the trial register

A treatment strategy research of chronic hepatitis B based on the central immune organs.

Trial registration number

ChiCTR-IOR-14005266.

Acknowledgements

The study was funded in full by grants from the twelfth

Five-Year major science and technology projects (2014ZX10002002-001-002).

Conflict of Interest

There are no conflicts of interest in this study.

Author's contribution

All work was done by the author named in this article and the authors accept all liability resulting from claims which relate to this article and its contents. The study was conceived and designed by Jun Lv; Yanpin Ma, Xuli Bao, Fang Xiong, Jinhuan Wang, Na Gu, Jia Guo, Huili Wu and Jun Lv collected and analysed the data; Yanpin Ma wrote the text and all authors have read and approved the text prior to publication.

References

1. Stanaway JD, Flaxman AD, Naghavi M, Fitzmaurice C, Vos T, Abubakar I, et al. The global burden of viral hepatitis from 1990 to 2013: findings from the Global Burden of Disease Study 2013. The Lancet 2016; 388: 1081-1088.
2. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol 2017; 67: 370-398.
3. Levrero M, Pollicino T, Petersen J, Belloni L, Raimondo G, Dandri M. Control of cccDNA function in hepatitis B virus infection. J Hepatol 2009; 51: 581-592.
4. Wooddell CI, Yuen MF, Chan HL, Gish RG, Locarnini SA, Chavez D, et al. RNAi-based treatment of chronically infected patients and chimpanzees reveals that integrated hepatitis B virus DNA is a source of HBsAg. Sci Transl Med 2017; 9: 409.
5. Mason WS, Gill US, Litwin S, Zhou Y, Peri S, Pop O, et al. HBV DNA Integration and Clonal Hepatocyte Expansion in Chronic Hepatitis B Patients Considered Immune Tolerant. Gastroenterol 2016; 151: 986-998.
6. Lindh M, Rydell GE, Larsson SB. Impact of integrated viral DNA on the goal to clear hepatitis B surface antigen with different therapeutic strategies. Curr Opin Virol 2018; 30: 24-31.
7. Rehermann B, Bertoletti A. Immunological aspects of antiviral therapy of chronic hepatitis B virus and hepatitis C virus infections. Hepatol 2015; 61: 712-721.
8. Thimme R, Dandri M. Dissecting the divergent effects of interferon-alpha on immune cells: time to rethink combination therapy in chronic hepatitis B? J Hepatol 2013; 58: 205-209.
9. Maini MK, Gehring AJ. The role of innate immunity in the immunopathology and treatment of HBV infection. J Hepatol 2016; 64: 60-70.
10. Bertoletti A, Ferrari C. Innate and adaptive immune responses in chronic hepatitis B virus infections: towards restoration of immune control of viral infection. Gut 2012; 61: 1754-1764.
11. Yang HC, Kao JH. Viral hepatitis. HBV cure--can we pin our hopes on immunotherapy? Nat Rev Gastroenterol Hepatol 2015; 12: 129-131.
12. Li GJ, Yu YQ, Chen SL, Fan P, Shao LY, Chen JZ, et al. Sequential combination therapy with pegylated interferon leads to loss of hepatitis B surface antigen and hepatitis B e antigen (HBeAg) seroconversion in HBeAg-positive chronic hepatitis B patients receiving long-term entecavir treatment. Antimicrob Agents Chemother 2015; 59: 4121-4128.
13. Ning Q, Han M, Sun Y, Jiang J, Tan D, Hou J, et al. Switching from entecavir to PegIFN alfa-2a in patients with HBeAg-positive chronic hepatitis B: a randomised open-label trial (OSST trial). J Hepatol 2014; 61: 777-784.
14. Loggi E, Gramenzi A, Margotti M, Cursaro C, Galli S, Vitale G, et al. In vitro effect of thymosin-alpha1 and interferon-alpha on Th1 and Th2 cytokine synthesis in patients with eAg-negative chronic hepatitis B. J Viral Hepat 2008; 15: 442-448.
15. King R, Tuthill C. Immune Modulation with Thymosin Alpha 1 Treatment. Vitam Horm 2016; 102: 151-178.
16. Serafino A, Pierimarchi P, Pica F, Andreola F, Gaziano R, Moroni N, et al. Thymosin alpha1 as a stimulatory agent of innate cell-mediated immune response. Ann N Y Acad Sci 2012; 1270: 13-20.
17. Naylor PH, Mutchnick MG. Immunotherapy for hepatitis B in the direct acting antiviral era: Reevaluating the thymosin alpha1 efficacy trials in the light of a combination therapy approach. J Viral Hepat 2018; 25: 4-9.
18. European Association For The Study Of The L. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol 2012; 57: 167-185.
19. Lucifora J, Xia Y, Reisinger F, Zhang K, Stadler D, Cheng X, et al. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. Sci 2014; 343: 1221-1228.
20. Belloni L, Allweiss L, Guerrieri F, Pediconi N, Volz T, Pollicino T, et al. IFN-alpha inhibits HBV transcription and replication in cell culture and in humanized mice by targeting the epigenetic regulation of the nuclear cccDNA minichromosome. J Clin Invest 2012; 122: 529-537.
21. Xie Q, Zhou H, Bai X, Wu S, Chen JJ, Sheng J, et al. A randomized, open-label clinical study of combined pegylated interferon Alfa-2a (40KD) and entecavir treatment for hepatitis B "e" antigen-positive chronic hepatitis B. Clin Infect Dis 2014; 59: 1714-1723.
22. Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, et al. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005; 352: 2682-2695.
23. Thompson AJ, Nguyen T, Iser D, Ayres A, Jackson K, Littlejohn M, et al. Serum hepatitis B surface antigen and hepatitis B e antigen titers: disease phase influences correlation with viral load and intrahepatic hepatitis B virus markers. Hepatol 2010; 51: 1933-1944.
24. Marcellin P, Ahn SH, Ma X, Caruntu FA, Tak WY, Elkashab M, et al. Combination of Tenofovir Disoproxil Fumarate and Peginterferon alpha-2a Increases Loss of Hepatitis B Surface Antigen in Patients With Chronic Hepatitis B. Gastroenterol 2016; 150: 134-144.
25. Brouwer WP, Sonneveld MJ, Xie Q, Guo S, Zhang N, Zeuzem S, et al. Peginterferon add-on results in more HBsAg decline compared to monotherapy in HBeAg-positive chronic hepatitis B patients. J Viral Hepat 2016; 23: 419-426.
26. Ning Q, Wu Z, Han H. Difficult-to-treat chronic hepatitis B. Infect Dis Inf 2011; 2: 2.
27. Lau GK. Current treatments for patients with HBeAg-positive chronic hepatitis B virus infection: a comparison focusing on HBeAg seroconversion. Liver Int 2010; 30: 512-520.
28. Chevaliez S, Hezode C, Bahrami S, Grare M, Pawlotsky JM. Long-term hepatitis B surface antigen (HBsAg) kinetics during nucleoside/nucleotide analogue therapy: finite treatment duration unlikely. J Hepatol 2013; 58: 676-683.
29. Nguyen T, Thompson AJ, Bowden S, Croagh C, Bell S, Desmond PV, et al. Hepatitis B surface antigen levels during the natural history of chronic hepatitis B: a perspective on Asia. J Hepatol 2010; 52: 508-513.
30. Jaroszewicz J, Calle Serrano B, Wursthorn K, Deterding K, Schlue J, Raupach R, et al. Hepatitis B surface antigen (HBsAg) levels in the natural history of hepatitis B virus (HBV)-infection: a European perspective. J Hepatol 2010; 52: 514-522.
31. Seto WK, Wong DK, Fung J, Huang FY, Lai CL, Yuen MF. Reduction of hepatitis B surface antigen levels and hepatitis B surface antigen seroclearance in chronic hepatitis B patients receiving 10 years of nucleoside analogue therapy. Hepatol 2013; 58: 923-931.

32. Lin TC, Chiu YC, Chiu HC, Liu WC, Cheng PN, Chen CY, et al. Clinical utility of hepatitis B surface antigen kinetics in treatment-naive chronic hepatitis B patients during long-term entecavir therapy. World J Gastroenterol 2018; 24: 725-736.
33. Sprengers D, Janssen HL. Immunomodulatory therapy for chronic hepatitis B virus infection. Fundam Clin Pharmacol 2005; 19: 17-26.
34. Wherry EJ, Blattman JN, Ahmed R. Low CD8 T-cell proliferative potential and high viral load limit the effectiveness of therapeutic vaccination. J Virol 2005; 79: 8960-8968.
35. Wherry EJ, Ahmed R. Memory CD8 T-cell differentiation during viral infection. J Virol 2004; 78: 5535-5545.
36. Tan AT, Hoang LT, Chin D, Rasmussen E, Lopatin U, Hart S, et al. Reduction of HBV replication prolongs the early immunological response to IFNalpha therapy. J Hepatol 2014; 60: 54-61.
37. Boni C, Penna A, Ogg GS, Bertoletti A, Pilli M, Cavallo C, et al. Lamivudine treatment can overcome cytotoxic T-cell hyporesponsiveness in chronic hepatitis B: new perspectives for immune therapy. Hepatol 2001; 33: 963-971.
38. Boni C, Penna A, Bertoletti A, Lamonaca V, Rapti I, Missale G, et al. Transient restoration of anti-viral T cell responses induced by lamivudine therapy in chronic hepatitis B. J Hepatol 2003; 39: 595-605.
39. Carey I, D'Antiga L, Bansal S, Longhi MS, Ma Y, Mesa IR, et al. Immune and viral profile from tolerance to hepatitis B surface antigen clearance: a longitudinal study of vertically hepatitis B virus-infected children on combined therapy. J Virol 2011; 85: 2416-2428.
40. Jiang Y, Li W, Yu L, Liu J, Xin G, Yan H, et al. Enhancing the antihepatitis B virus immune response by adefovir dipivoxil and entecavir therapies. Cell Mol Immunol 2011; 8: 75-82.
41. Nguyen KB, Salazar-Mather TP, Dalod MY, Van Deusen JB, Wei Xq, Liew FY, et al. Coordinated and Distinct Roles for IFN- , IL-12, and IL-15 Regulation of NK Cell Responses to Viral Infection. J Immunol 2002; 169: 4279-4287.
42. Micco L, Peppa D, Loggi E, Schurich A, Jefferson L, Cursaro C, et al. Differential boosting of innate and adaptive antiviral responses during pegylated-interferon-alpha therapy of chronic hepatitis B. J Hepatol 2013; 58: 225-233.
43. Serafino A, Pica F, Andreola F, Gaziano R, Moroni N, Moroni G, et al. Thymosin alpha1 activates complement receptor-mediated phagocytosis in human monocyte-derived macrophages. J Innate Immun 2014; 6: 72-88.
44. Yang YF, Zhao W, Zhong YD, Yang YJ, Shen L, Zhang N, Huang P. Comparison of the efficacy of thymosin alpha-1 and interferon alpha in the treatment of chronic hepatitis B: a meta-analysis. Antiviral Res 2008; 77: 136-141.
45. Kim BH, Lee YJ, Kim W, Yoon JH, Jung EU, Park SJ, et al. Efficacy of thymosin alpha-1 plus peginterferon alpha-2a combination therapy compared with peginterferon alpha-2a monotherapy in HBeAg-positive chronic hepatitis B: a prospective, multicenter, randomized, open-label study. Scand J Gastroenterol 2012; 47: 1048-1055.