A randomized, placebo-controlled trial of thymosin-α1 and lymphoblastoid interferon for HBeAg-positive chronic hepatitis B

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Combination therapy between two immunomodulators used for treatment of chronic hepatitis B was explored based on reported therapeutic efficacy of interferon-α, and thymosin-α1 as monotherapeutic agents to determine if combination therapy was superior to interferon alone. This double-blinded, randomized, placebo-controlled trial compares the addition of thymosin-α1, 1.6 μg taken three times per week (combination therapy) or thymosin placebo (monotherapy) to lymphoblastoid interferon (Wellferon®), 5 million international units (MIU) taken three times per week, for 24 weeks. Entry criteria included positive hepatitis B e antigen (HBeAg); alanine aminotransferease (ALT) ≥1.5× upper normal limit, but ≤10 × upper normal limit; positive HBV DNA; absence of cirrhosis; treatment naivety and no co-morbid factors. A total of 98 HBeAg-positive patients were recruited, of which 48 were randomized to combination therapy and 50 to monotherapy. The primary endpoint was the loss of HBeAg at 72 weeks. The secondary endpoints were HBeAg seroconversion, normalization of ALT, loss of HBV DNA and improvement in histology. The HBeAg loss was 45.8% and 28.0% for combination therapy and monotherapy, respectively (difference, 17.8%; 95% CI -1.2%–35.3%, *P*=0.067). There was a trend towards HBeAg loss when using combination therapy. There were also no statistically significant differences between the different therapies with respect to the secondary endpoints of HBeAg seroconversion, changes in histology, normalization of ALT or loss of HBV DNA. In conclusion, this trial showed a 17.8% improvement in HBeAg loss rates using combination therapy over interferon monotherapy. This could clinically indicate a potential important difference that would need confirmation in subsequent trials.

Introduction

Therapeutic options for chronic hepatitis B (CHB) was revolutionized by the introduction of nucleoside analogues, which suppress hepatitis B virus (HBV) DNA by more than 3 logs [1,2]. However, the therapeutic response measured by hepatitis B e antigen (HBeAg) seroconversion appears suboptimal, occurring in only 16% of patients after 48 weeks of lamividine treatment [1], and 12% (HBeAg loss 24%) after 48 weeks of adefovir treatment [2]. Meta-analysis of interferon treatment against HBeAg-positive CHB estimates rate of HBeAg loss as 33% [3], but direct comparisons of the efficacy of interferon to lamivudine show no difference in HBeAg seroconversion rates between interferon or lamivudine monotherapy [4].

Another immunomodulator, thymosin-α1, has also been evaluated in patients with both HBeAg-positive and -negative CHB, but the therapeutic results are variable, with HBeAg clearance rates among patients with HBeAg-positive CHB 1 year after thymosin therapy ranging from 25% to 40.9% [5–6]. One randomized controlled trial from the United States reported no benefit in patients with HBeAg-positive CHB [5],

although a trend towards delayed efficacy was seen. Yet another trial in Taiwan showed a significant delayed virological response seen 1 year after stopping treatment [6]. Such delayed virological response was also seen in trials in patients with HBeAg-negative CHB, with an odds ratio of 2.69 at 1 year post-therapy in a meta-analysis on thymosin trials [7].

The limited efficacy of existing monotherapy has led to the exploration of combination therapy. Currently the ideal combination of therapeutic agents is still under exploration. Prior to the start of our trial, the combination of two immunomodulators had only been assessed in a small open-labelled study on 15 anti-HBe (antibodys for HBeAg)-positive CHB patients, which showed promizing results with nine patients (60%) undergoing some virological response (loss of HBV DNA), and six (40%) losing HBeAg [8].

Thus, the current trial was designed as a double–blinded, randomized, placebo-controlled trial examining the combination of two immunomodulators for the treatment of HBeAg-positive CHB versus interferon monotherapy and to confirm the promising results of Rasi *et al.* [8]. The rationale for combining two immunodulators comes from the pathogenesis of CHB itself. Hepatitis B is thought to be non-pathogenic, and liver necroinflammatory disease is a result of the host's immune responses against viral antigens [9].

Materials and methods

Patients

Treatment-naive patients with HBeAg-positive CHB of either gender aged over 16 years were eligible to enter the trial based on the following criteria: positive hepatitis B surface antigen (HBsAg) for more than 6 months, positive HBeAg, alanine aminotransferease (ALT) between 1.5-times and 10-times the upper limit of normal (ULN), positive HBV DNA by a non-PCR method, absence of hepatitis C, delta or HIV infection, absence of decompensated liver disease, absence of complete cirrhosis on liver biopsy, absence of significant medical co-morbidities including malignancy and recent treatment with steroids or immunosuppresants. All patients were also required to have haemoglobin ≥ 10 g/dl, platelet count ≥ 75,000 mm^3, white cell count ≥ 3,000 mm^3, normal serum creatinine, prothrombin time (PT)<4 s prolonged over control values, normal serum bilirubin, and serum albumin ≥ 30 g/dl.

Study design

The trial was designed as a multicentre 1:1 randomized placebo-controlled trial comparing 5 million international units (MIU) of lymphoblastoid interferon (Wellferon®, GlaxoWellcome, Beckenham, UK) and 1.6 μg of thymosin-α1 (SciClone Pharmaceuticals, Inc., San Mateo, CA, USA) both taken three times weekly (combination therapy) against 5 MIU of lymphoblastoid interferon and thymosin placebo both taken three times weekly (monotherapy). The treatment period was 24 weeks with a follow-up period of 48 weeks. The interferon dose of 5 MIU to be taken three times weekly was based on the recommended dosage in the regional guidelines [10,11]. Liver biopsies were performed at baseline and at the end of follow-up, in other words week 72 of the trial. The trial was conducted at the National University Hospital (NUH), Changi General Hospital, Tan Tock Seng Hospital, Singapore and at the Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. The trial protocol was approved by the Ethics Committees of all participating hospitals and written informed consent was obtained from all patients. Trial monitoring and data management were performed by the Clinical Trials and Epidemiology Research Unit (CTERU), Singapore. Laboratory tests were performed at the individual hospital laboratories, but HBV DNA testing was centralized at the Molecular Diagnostic Laboratory at NUH, Singapore. All histologies were read by a single pathologist (AW). Hepatic necroinflammation and fibrosis were assessed by a standard scoring system [12]. Wellferon® was purchased commercially from GlaxoWellcome and thymosin-α1 or the placebo was provided as the label blinded trial drug by SciClone Pharmaceuticals. All investigators, research staff, laboratory staff, pathologists and data entry staff were blinded to the drug allocation during the trial period. Compliance to therapy was assessed by counting returned vials of interferon and thymosin or placebo.

Randomization

Once eligibility had been confirmed and written informed consent obtained, patients were randomly assigned to receive combination therapy or monotherapy through the central randomization office of the CTERU. Patients were entered into the trial based on a 1:1 treatment allocation stratified by hospital, HBV DNA (<100 or ≤100 pg/ml) and HAI (hepatic activity index) score (6–13 or 14–20).

Study visits

During the initial month, patients were seen weekly to monitor their blood counts, following which they were seen every 4 weeks until the end of their treatment (24 weeks after trial entry). Patients were then seen every 8 weeks during the 48 week follow-up period, during which time they were examined, and had blood taken for full blood counts and serum biochemistry. HBV DNA tests were also done at every visit. HBeAg and anti-HBe tests were performed at week 24 and week 72, but if either result was negative,

HBeAg and anti-HBe were tested in all the patient's samples throughout the trial period.

Study endpoints

The primary endpoint was loss of HBeAg at 72 weeks post-randomization. Secondary endpoints included HBeAg seroconversion (the loss of HBeAg with the development of anti-HBe), normalization of ALT, improvement in hepatic necroinflammation (histological response) and a drop of HBV DNA below 5 log copies/ml. These were evaluated at the end of treatment and at week 72, whereas the patient's histological response was examined at week 72. The times taken to achieve HBeAg loss, HBeAg seroconversion or normal ALT were also determined.

Tests

The HBV DNA assay was performed by two methods. To determine the loss of HBV DNA, the Digene liquid hybridization assay was used (Digene Corp, Gaithersburg, MD, USA), with a lower limit of detection of 140,000 copies/ml. To analyse the changes in HBV DNA over time, HBV viral load was measured by the Bayer VERSANT HBV 3.0 Assay (bDNA) using the System 340 bDNA Analyzer (Bayer Diagnostics, Tarrytown, NY, USA). This amplification assay employs a series of sequential nucleic acid hybridization steps to produce a chemiluminescent signal, which is proportional to the amount of HBV DNA in the original sample. The results are reported in both copies/ml and international units (IU)/ml. The assay range is from 2.0×10^3 copies/ml to 1.0×10^8 copies/ml (357 IU/ml to 17,857,100 IU/ml).

HBV genotyping was performed after DNA extraction and amplification of the envelope region using the following sense primer: 5′-GCC TCA TTT TGT GGG TCA CCA TA-3′ (nucleotides [nt] 1408 to 1430) and the following anti-sense primer 5′-TCT CTG ACA TAC TTT CCA AT-3′ (nt 2817 to 2798). After 40 rounds of PCR, a further semi-nested round of amplification was performed using 2μl of the first round product as template, an internal sense primer 5′-TGG GGG TGG AGC CCT CAG GCT-3′ (nt 1676 to 1696) and the original anti-sense primer. The amplification conditions were reduced to 25 rounds of cycling. The deduced sequences were compared with published sequences to confirm genotype [13].

Safety

All patients who received at least one dose of trial medication were evaluated for adverse events during treatment and follow-up. Clinical and laboratory abnormalities were graded according to severity score based on the Common Toxicology Criteria of the National Institutes of Allergy and Infectious Diseases [14].

Patients with any moderate constitutional adverse events such as nausea or lethargy underwent a 50% dose reduction of interferon, whereas patients with severe constitutional adverse events had their interferon stopped.

Interferon dosage was also halved in patients with granulocytes count less than 0.75×10^9/l or if the platelet count was less than 5×10^{10}/l. Interferon was stopped when granulocytes were less than 0.5×10^9/l or platelets were less than 3×10^{10}/l.

Statistical considerations

At the time of trial design in 1997, there was little data on the efficacy of thymosin and even less on the efficacy of combination therapy. Virological response from monotherapy in one meta-analysis was 33% [3], whereas combination therapy produced a virological response of 60% in the first pilot study [8]. Hence, we anticipated a 30% difference in response between the two treatment arms. With a two-sided test size of 5% and a power of 85%, an estimated sample size of 48 patients was required in each randomization arm [15].

Analysis was done on an intention–to–treat (ITT) basis. Comparisons of all categorical variables were performed using Chi-square tests except for ALT normalization and HBV DNA loss, which were compared by using logistic regression with adjustment for their respective baselines. For binary endpoints, missing values were treated as failure. The comparisons of HAI score and fibrosis score between baseline and end of follow-up were performed by using paired *t*-tests, whereas those scores at week 72 were compared by using linear regression adjusting for their respective baseline. The 95% confidence intervals (CIs) for the treatment differences of binary and continuous outcomes were calculated using CIA statistical software [16].

The time to HBeAg loss and ALT normalization was determined by Kaplan–Meier survival analysis and were compared by log rank tests.

The trial data was collected on printed forms, and entered into CLINTRIAL™ 4 release 4.2 [17]. The statistical analysis was performed using SPSS version 11.5 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

A total of 98 patients from three hospitals in Singapore and one in Bangladesh were enrolled into the trial between 28 July 1997 and 25 July 2001, with the last follow-up on 19 December 2002. Forty-eight patients were assigned to combination therapy, and 50 to

monotherapy. The mean age of the patients was 30.5 (range, 17–60) years, 76% were males, 71% ethnically Chinese and 20% Bangladeshi. Patients in the two groups were comparable in terms of demographic and baseline characteristics including serum ALT level, HBV DNA level and genotype distribution (Table 1). All but two patients completed trial medication; one receiving monotherapy discontinued after 3 weeks of therapy due to significant cytopenia and one receiving combination therapy discontinued at week 12 after developing optic neuritis. Only six patients (two monotherapy patients and four combination therapy patients) had a reduction in their interferon dose due to reported adverse events, while five patients (three monotherapy and two combination) were lost to follow-up (Figure 1).

Virological response

At the end of treatment (week 24) only a 3% advantage in HBeAg loss in combination therapy patients over monotherapy patients was noted. However, by week 72, a trend towards significance was seen with 22 (45.8%) combination therapy patients demonstrating HBeAg loss, compared to 14 (28.0%) patients on monotherapy, a difference of 17.8% (95% CI, –1.2%–35.3%; P=0.067; Table 2).

HBeAg seroconversion occurred in 21 (43.8%) patients on combination therapy compared to 14 (28.0%) patients on monotherapy at 72 weeks (a difference of 15.8%; 95% CI, -3.1%–33.3%; P=0.104). There were no significant differences in normalization of ALT or loss of HBV DNA (Table 2). Neither were there significant differences in time to reach HBeAg loss (Figure 2A), HBeAg seroconversion or normal ALT (Figure 2B).

Four combination therapy patients with HBeAg seroconversion continued to have high titre of HBV DNA level, ranging from 42 to 2,572 pg/ml, with serum ALT level ranging from 40 IU/l to 551 IU/l. All of their liver biopsies at end of follow-up showed active inflammation with HAI scores ranging from 15 to 20. They were all considered to have developed HBeAg-negative CHB. All four patients had been randomized to receive combination therapy.

Histological response

Although the overall mean HAI score decreased by 1.99 from 10.50 to 8.82 by week 72, there was no significant difference in improvement between patients receiving combination therapy and monotherapy (P=0.712; Table 2). There was only a small increase of 0.11 in mean fibrosis over the same period, and also little difference between the two treatment groups (P=0.863; Table 2).

Hepatitis B flares

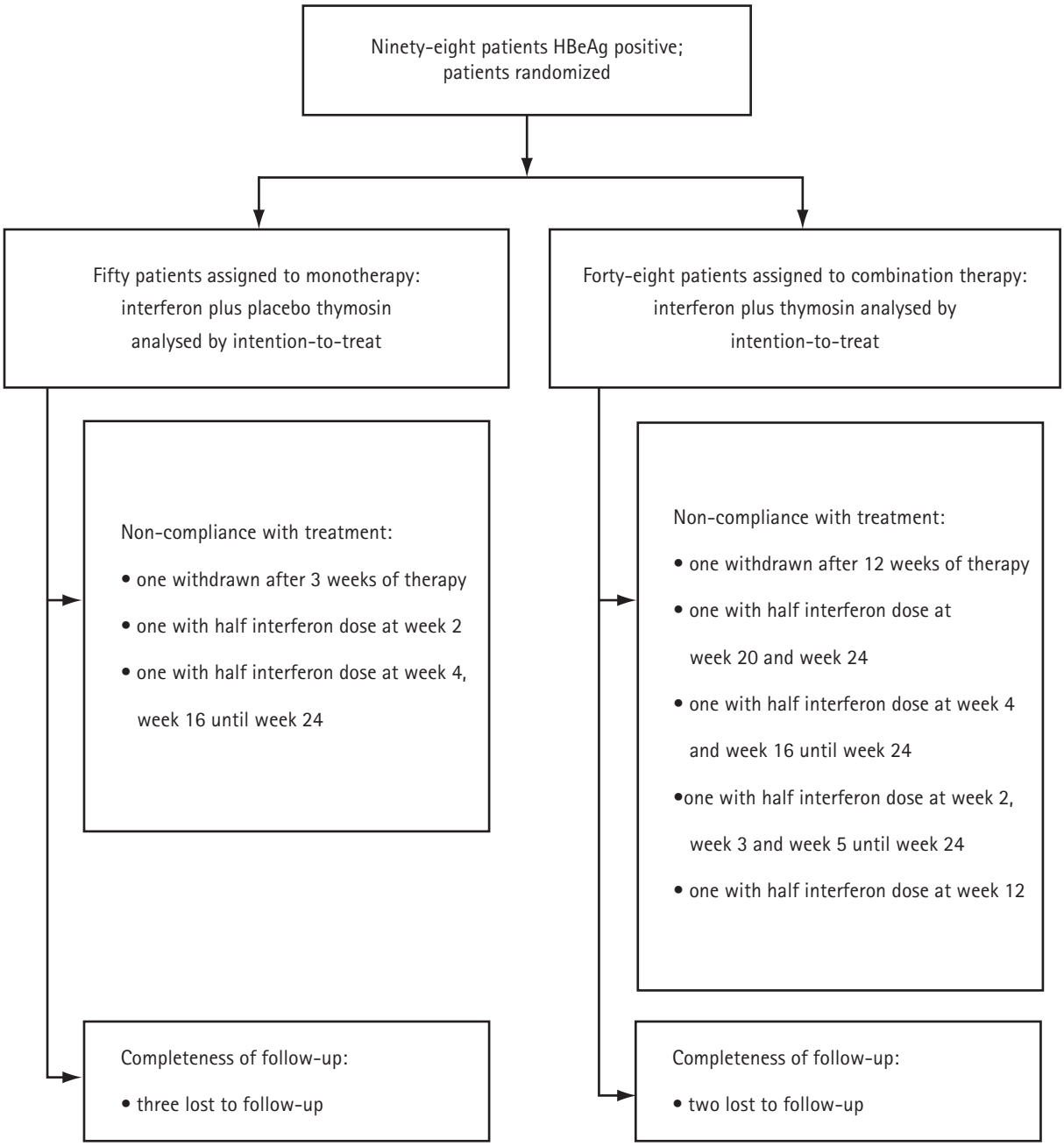
Twelve combination therapy (25.0%) patients developed hepatitis B flares (defined as ALT ≥5 times ULN) compared to 14 (28.0%) patients on monotherapy (difference, –3.0%; 95% CI –20.0% to 14.3%; P=0.737). Twenty patients developed flares during follow-up (8 combination therapy and

Table 1. Patients' baseline characteristics by treatment group

	Combination (n=48)	Monotherapy (n=50)
Male (%)	35 (73)	39 (78)
Age, year*	32.3 (19–60)	28.9 (17–46)
Ethnicity, %		
Chinese	34 (71)	36 (72)
Bangladeshi	10 (21)	10 (20)
Malay	4 (8)	3 (6)
Indian	0 (0)	1 (2)
Weight, kg*	62.1 (42–100)	64.2 (41.7–115.2)
Body-mass index*	22.1 (16.3–31.6)	22.9 (17.6–38.9)
Bilirubin, umol/l*	11.6 (0.6–42.0)	11.5 (0.6–25)
ALT, × U/l*	219.1 (49–1,598)	188.3 (30–795)
AST, × U/l*	123.0 (33–846)	90.5 (17–320)
HBV DNA titre, pg/ml†	956 ± 269	737 ± 222
HBV genotype		
B	18 (38)	21 (42)
C	20 (42)	23 (46)
Others	10 (20)	6 (12)
HAI score*	10.7 (2–20)	10.3 (1–20)
Fibrosis score*	2.2 (0–8)	2.4 (0–9)

*Results are displayed as mean (range). †Results displayed as mean ± standard error of mean. ALT, alanine aminotransferase; AST, aspartate aminotransferase; HAI, hepatic activity index; HBV, hepatitis B virus.

Figure 1. Trial profile



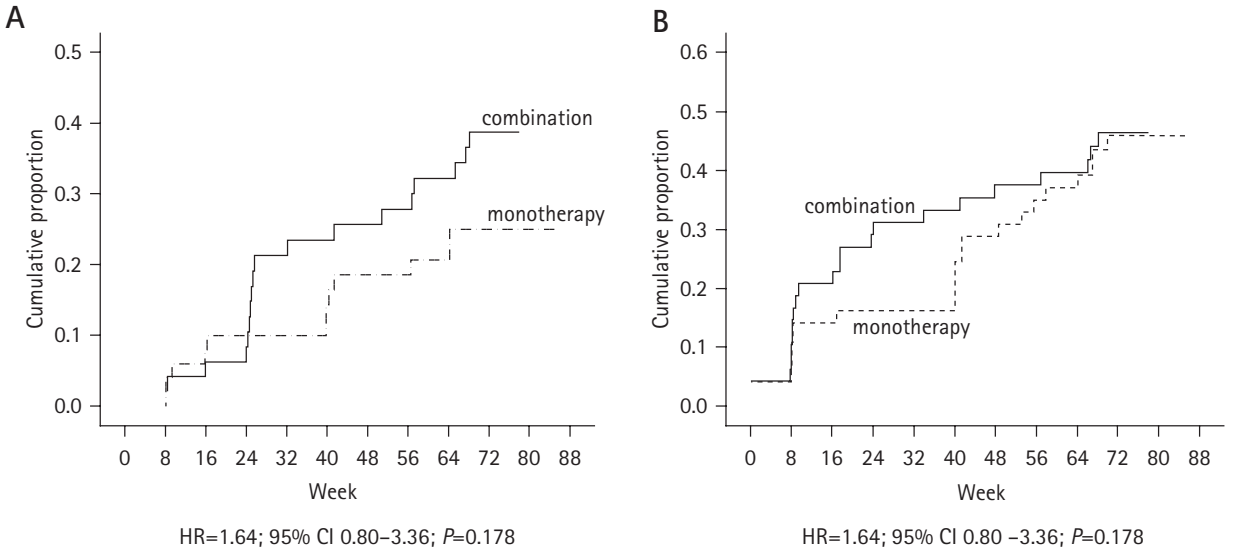
Consort diagram showing allocation and follow-up of patients in the study HBeAG, Hepatitis B e antigen.

12 monotherapy), but all cases were spontaneously resolved without hepatic decompensation and without alteration in therapy.

Serious adverse events (SAEs)

A total of 11 SAEs were reported from eight patients (four combination therapy and four monotherapy). Two patients were admitted for severe headache, but neither was found to have any significant neurological deficit and both resolved with analgesia. Two patients were admitted with fractures, one with a fractured fifth metacarpal after a box fell on his hand, and another had a fracture of the radius after a fall. Three patients were diagnosed as having biliary colic and/or cholecystitis,

Figure 2. Cumulative times to HBeAg loss, HBeAg seroconversion, and normal ALT



(A) A trend towards delayed HBeAg loss after treatment was seen in the Combination group. Kaplan Meier curve of time to HBeAg seroconversion is similar to **(A)**. **(B)** There is a trend towards normalization of ALT in Combination group at the end of treatment but this difference was lost by the end of follow up. ALT, alanine aminotransferase; CI, confidence interval, HBeAG, hepatitis B e antigen; HR, hazard ratio.

two were discharged after resolution of the acute event, whereas the remaining patient proceeded to a cholecystectomy. Two patients were admitted for fever, one from urinary tract infection and the other from infective gastroenteritis, but the fevers receded after treatment with antibiotics. One monotherapy patient was admitted with a grade 3 hepatitis B flare with jaundice in the follow-up period, but his flare resolved

Table 2. Virological and histological responses by treatment group at end of treatment and follow-up

Outcome	Combination (C) *n*=48	Monotherapy (M) *n*=50	Difference (C–M) (95% CI)	*P*-value
End of treatment (%)				
HBeAg loss	12 (25.0)	11 (22.0)	3.0 (-13.6–19.6)	0.726
HBeAg seroconversion	12 (25.0)	11 (22.0)	3.0 (-13.6–19.6)	0.726
Normalization of ALT	31 (64.6)	26 (52.0)	12.6 (-6.8–30.6)	0.207*
Loss of HBV DNA (LLD 140,000 copies/ml)	22 (45.8)	18 (36.0)	9.8 (-9.4–28.1)	0.655*
Loss of HBV DNA (LLD 2,000 copies/ml)	4 (8.3)	3 (6)	0.3 (-11.6–12.6)	0.871*
End of follow up (%)				
HBeAg loss	22 (45.8)	14 (28.0)	17.8 (-1.2–35.3)	0.067
HBeAg seroconversion	21 (43.8)	14 (28.0)	15.8 (-3.1–33.3)	0.104
Normalization of ALT	27 (56.3)	28 (56.0)	0.3 (-18.7 to 19.2)	0.982*
Loss of HBV DNA (LLD 140,000 copies/ml)	18 (37.5)	18 (36.0)	1.5 (-17–20)	0.836*
Loss of HBV DNA (LLD 2,000 copies/ml)	6 (12.5)	4 (8.0)	4.5 (-8.2–17.6)	0.480*
Loss of HBsAg	0	0		
Histological response				
HAI score				
Mean (SD)	8.69 (6.69)	8.95 (5.92)	-0.26 (-3.09–2.57)	0.712*
Range	1.0–20.0	1.0–20.0		
Fibrosis score				
Mean (SD)	2.24 (2.24)	2.55 (1.97)	-0.31 (-1.20–0.58)	0.683*
Range	0.0–6.0	0.0–6.0		

ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg , hepatitis B surfact antigen; HAI, hepatic activity index; HBV, hepatitis B virus; LLD, lower limit of detection.

spontaneously without any clinical decompensation. One combination therapy patient developed acute reduced vision in one eye and was diagnosed as having retrobulbar neuritis. The trial medication was discontinued immediately, and the patient was treated with steroids and non-steroidal anti-inflammatory agents as well as lamivudine. Although the patient's vision gradually improved it was not back to normal 10 months after stopping the trial medication. Only this SAE was thought to be related to trial medication.

Adverse events (AEs)

The mean AE rate in the combination therapy arm was 11.29 per patient and 11.68 per patient in the monotherapy arm. The top ten AEs in combination therapy patients were fever, tiredness, muscle aches, nausea, headache, hair loss, anorexia, flu symptoms, sore throat and vomiting; whereas for monotherapy patients they were fever, tiredness, flu symptoms, headache, muscle aches, anorexia, hair loss, nausea, increased ALT/aspartate aminotransferase (AST) and sore throat. There was no difference in the types of AE seen between the two groups other than increased ALT/AST, which was seen in 17 (34.0%) monotherapy patients (18 events) compared to 5 (10.4%) combination therapy patients (5 events; P=0.005).

Discussion

This trial failed to confirm the promising results of a pilot study performed by Rasi *et al.* [8]. In their open-labelled study, 15 patients with anti-HBe positive hepatitis B, some of whom had failed interferon monotherapy, were treated with a combination of lymphoblastoid interferon (3 MIU) and thymosin-α1 (1 mg) twice weekly after a short period of 'induction' therapy. Nine out of the 15 patients (60%) lost HBV DNA and the remaining six patients (40%) lost HBsAg at the end of 48 weeks of post-treatment follow-up. In another recent non-randomized study, Saruc [18] enrolled 21 anti-HBe-positive patients who were treated for 26 weeks with a combination of 10 MIU of interferon-α taken three times weekly and 1.6 mg thymosin-α1 taken twice weekly and then further interferon-α, at the same dosage, for a further 26 weeks. After 26 weeks of post-treatment follow-up, 21 patients (76.2%) were sustained responders to therapy with loss of HBV DNA and normalization of ALT. The differences between these small open-labelled studies and our trial can be explained by potential patient selection bias from non-randomized studies, and the use of anti-HBe positive patients who may have a different natural history and response to therapy than HBeAg-positive patients.

While our randomized trial did not show any statistically significant benefit of interferon and thymosin combination therapy over interferon monotherapy, there was a trend towards improvement at the end of follow-up with regard to HBeAg loss showing a treatment difference of 17.8% (95% CI 1.2%–35.3%). The sample size calculations we made when planning this randomized trial was based on information from the small non-randomized study by Rasi *et al.* [8]. The study suggested the addition of thymosin to interferon could increase response rates from approximately 30% to 60% (effect size=30%). Our trial estimate of 17.8% from 98 patients suggests that the 'true' effect size is more likely to be in the range of 15% to 20%. Utilizing our study's baseline rate of 28% from the monotherapy arm, a test size of 5%, a power of 85% and taking a conservative effect size of 15%, a randomized control trial (1:1) would require 328 patients. Thus larger studies would be needed to confirm our findings. This treatment effect of thymosin seems to be consistent with the study by Mutchnick *et al.* [5], rather than that of Chien *et al.* [6] with regards to thymosin monotherapy. The Kaplan–Meier analysis of HBeAg seroconversion showed a non-significant trend towards delayed response in those treated with combination therapy compared to those given interferon alone, similar to previous reports of the delayed effects of thymosin [6].

A greater effect may have been achieved with more optimal drug dosing. Higher doses of interferon appear to be more efficacious [19], and more recently, the use of pegylated interferon has significantly increased therapeutic efficacy [20]. We have used a more frequent dosing for thymosin than has been reported in previous clinical trials, as the optimal dose of thymosin remains unclear [21]. Consequently it remains to be seen whether combination therapy with higher doses of interferon or pegylated interferon, and more optimal thymosin dosing can provide a better therapeutic response. Notably, the benefits of thymosin and its mechanism of action could be different in anti-HBe-positive patients.

Although there were a high number of serious adverse events, only optic neuritis was attributed to the treatment. The higher rate of adverse events is typical of interferon therapy and is the main limiting factor of interferon therapy [22]. Thymosin did not appear to be associated with any AEs or SAEs.

The rationale behind this double-blinded randomized trial was the different postulated mechanism of action of interferon and thymosin. Interferon acts through diverse mechanisms, which includes the upregulation of the MxA protein [23,24], a proteasome-dependent pathway [25], through cytokine release [26] and the 2–5A oligosynthetase system [27], which is likely to have multiple modes of action [27]. Thymosin has been reported to increase T-lymphocyte function (especially T-cell maturation and enhanced

cytotoxicity), natural-killer cells, and B-lymphocyte antibody production [28,29].

So far, studies combining nucleoside analogues have not shown to be advantageous over monotherapy [30,31], and the efficacy of combination lamivudine and interferon therapy has shown mixed results. The initial study [4] showed no advantage combining interferon and lamivudine over monotherapy by intention-to-treat analysis. Subsequently, an Italian study [32] showed a significant difference in combination therapy (33% sustained HBeAg seroconversion) over lamivudine monotherapy (15% sustained HBeAg seroconversion), but for patients who had failed interferon therapy [33], combination therapy for 24 weeks was surprisingly worse based on HBeAg loss (21% sustained HBeAg seroconversion) than lamivudine monotherapy for 48 weeks (33% sustained HBeAg seroconversion). Recently, the combination of pegylated interferon and lamivudine has shown considerable promise in treatment of CHB [20,34,35] with high sustained response rates. Taken together, the current study provides important information in mapping the treatment of CHB. If indeed combination therapy is the future of CHB therapy [36], then it appears that new combinations of immunomodulators need to be explored that are more potent or have novel mechanisms of action, as existing combinations show no benefit over monotherapy. Bearing in mind the limitations of the existing study, the combination of two immunomodulators in the current trial has not shown a marked therapeutic benefit. In a small study of thymosin and famciclovir little benefit was seen [37]. Combination therapy in CHB has not fulfilled the promise seen in HIV and chronic hepatitis C, but further studies are needed to exploit optimal therapeutic strategies.

In conclusion, this in-principle double-blinded randomized trial has shown a 17.8% increased HBeAg loss and 15.8% increased HBeAg seroconversion in patents taking a combination of thymosin and interferon over patients receiving interferon monotherapy (a trend towards significance). This trend may be worth pursuing in larger clinical trials. It is possible that other more efficacious combinations of therapy may be more beneficial, but this remains to be explored.

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Conflict of interest statement

None of the authors had any conflicts of interests with regards to the study design, collection, analysis, in the interpretation of data, in writing the report and in the decision to submit the paper for publication. The sponsors of the study had no role in any of the above.

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