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Effectiveness of lamivudine and interferon-α combination therapy versus interferon-α monotherapy for the treatment of HBeAg-negative chronic hepatitis B patients: a randomized clinical trial

Oguz Karabay[1], Ali Tamer[2], Mustafa Tahtaci[2], Seref Vardi[2], Harika Celebi[2]

[1]Department of Clinical Microbiology and Infectious Diseases and [2]Department of Internal Medicine, Izzet Baysal University School of Medicine, Bolu, Turkey

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Results comparing the effectiveness of lamivudine used as monotherapy or in combination with interferon-alpha (IFN-α) in the treatment of chronic hepatitis B are not conclusive. This study compared the effects of IFN-α alone or in combination with lamivudine for the treatment of hepatitis B e antigen (HBeAg)-negative patients with chronic hepatitis B. Participation of patients in the IFN-α monotherapy and combination groups was randomized to a 1:1 ratio. Twenty seven HBeAg-negative patients with chronic hepatitis B received IFN-α (13 patients) at 9 million units 3 times weekly for 24 weeks or IFN-α at 9 million units 3 times weekly for 24 weeks plus lamivudine 100 mg/day (14 patients) daily for 1 year. Hepatitis B virus (HBV) DNA was measured quantitatively by real-time polymerase chain reaction at 0, 6, 12 and 18 months after the start of treatment. Sustained virologic response was defined as non-detectable serum HBV DNA 72 weeks after starting treatment. Sustained biochemical response was defined as normalization of alanine aminotransferase (ALT) values 72 weeks after starting treatment. The baseline characteristics of the 2 treatment groups were similar with respect to age, gender, ALT, HBV DNA levels and histologic diagnosis. Sustained biochemical responses were found at week 72 in 7 patients in each group (54% with IFN-α monotherapy and 50% with combination therapy) [$p>0.05$]. Sustained virologic responses were found at week 72 in 5 patients (38%) in the monotherapy and 7 patients (50%) in the combination therapy group ($p>0.05$). Combination therapy was not superior to IFN-α alone for the treatment of chronic hepatitis B. Combination treatment was associated with some disadvantages, such as additional cost. Lamivudine, on the other hand, may be more suitable for patients with cirrhosis, non-responders to IFN-α or in cases with contraindication for IFN-α.

Key words: Combination drug therapy, hepatitis B, hepatitis B antibodies, interferon-alpha, lamivudine

Hepatitis B infection is prevalent worldwide and a major health burden due to the associated complications of hepatic fibrosis, cirrhosis and hepatocellular carcinoma that occur in the context of chronic infection [1,2]. Immunization and greater public awareness have significantly decreased the incidence of new hepatitis B virus (HBV) infection, but the treatment of persons already infected remains an important international health concern [1-3]. In the Mediterranean basin, 30-80% of patients with chronic hepatitis B are hepatitis B e antigen (HBeAg)-negative, in contrast to 10-40% rates in Northern European countries and the United States. HBeAg-negative chronic hepatitis B usually runs a progressive course. The greatest problem with the treatment of HBeAg-negative patients with chronic hepatitis B is the high relapse rate. Their end-treatment response rates are similar to those of classic chronic hepatitis B patients, but after discontinuation of treatment most of them relapse. The available literature indicates that more than 80% of patients with HBeAg-negative chronic hepatitis B infection do not respond to the current approved therapies [4].

Until recently, interferon-alpha (IFN-α) was the only drug approved throughout the world for the treatment of chronic hepatitis B infection. Unfortunately, relapse with return of viremia and hepatitis occurs in up to 50% of cases [5]. Lamivudine has been approved for the treatment of chronic hepatitis B infection [6]. Administered orally once daily, lamivudine has been shown to be as effective as IFN-α in clearing HBeAg.

Corresponding author: Dr. Oguz Karabay, Department of Clinical Microbiology and Infectious Diseases, Izzet Baysal University School of Medicine, Golkoy, Bolu, Turkey.
E-mail: drkarabay@yahoo.com

Although a majority of patients taking lamivudine demonstrate improved liver histology, development of lamivudine-resistant HBV mutants is common, especially with prolonged use, and diminishes the effectiveness of treatment [2].

The number of investigations of combination therapy with IFN-α and lamivudine in the treatment of chronic hepatitis B treatment is limited and the results of these studies are controversial [7-10]. The present study investigated whether combination IFN-α and lamivudine is safe and effective, and whether it can provide additional therapeutic benefits compared with IFN-α monotherapy for the treatment of HBeAg-negative chronic hepatitis B patients.

Materials and Methods

Subjects

This study was performed between December 1999 and December 2001 in the Social Security Hospital Duzce in Turkey. Approval for the study was obtained from the local ethics committee. All patients provided written consent to participate. None of the patients had previously received IFN-α or any other antiviral therapy. Liver biopsy was obtained from all patients before the start of treatment.

The inclusion criteria were as follows: positive hepatitis B surface antigen and positive anti-HBeAg antibodies for at least 6 months; serum HBV DNA $>10^5$ copies/mL; serum alanine aminotransferase (ALT) above at least two-fold the normal range; and liver biopsy showing chronic hepatitis (necroinflammatory score >4). Exclusion criteria were as follows: history of allergy to IFN-α or lamivudine; psychiatric illness; decompensated cirrhosis; pregnancy; breast-feeding; and age under 17 or over 65 years. A positive result for any HBeAg, hepatitis D, human immunodeficiency virus or hepatitis C was also considered sufficient basis for exclusion. Patients with a history of antiviral drug or IFN-α use were also excluded.

Randomization

Twenty seven consecutive chronic HBV patients who were HBeAg-negative were randomized at a 1:1 ratio to receive either IFN-α monotherapy (13 patients) or IFN-α plus lamivudine treatment (14 patients). IFN-α was started in both groups at the dose of 9 million units IFN-α2a (Roferon-A; Hoffmann-La Roche, Basel, Switzerland) 3 times weekly for 24 weeks. The combination therapy group also received lamivudine 100 mg (Zeffix; GlaxoSmithKline) to be taken orally once daily for 1 year starting at the time of initiation of IFN-α2a treatment.

Follow-up

All patients were monitored for at least 6 months. Clinical evaluation and laboratory tests such as complete blood count, aspartate aminotransferase, ALT, bilirubin, alkaline phosphatase, γ-glutamyltransferase, and additional studies were performed as needed before the start of the therapy. Blood samples and liver biopsy specimens were obtained 2-3 week before the start of therapy. Physical examinations, routine biochemical tests and blood cell counts were performed every month during the study period. Serum samples were tested by enzyme-linked immunosorbant assay to detect hepatitis B markers (Abbott Laboratories, USA). HBV DNA was measured quantitatively at 0, 6, 12 and 18 months after the start of the study by real-time polymerase chain reaction with a lower limit of detection of 200 HBV DNA copies/mL, a reportable range of 200 to 200,000,000 HBV DNA copies/mL, and a reported linear range between 50 and 108 IU/mL (Roboscreen-TheBIO-Quantification Company, Leipzig, Germany).

Outcomes

Biochemical response was defined as the normalization of ALT values. Virologic response was defined as the loss of detectable serum HBV DNA. Sustained virologic response was defined as non-detectable serum HBV DNA at 72 weeks after starting treatment. Sustained biochemical response was defined as normalization of ALT values at 72 weeks after starting treatment. Additional tests were performed as needed.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences version 10.0 software. Quantitative variables of the 2 groups were expressed as mean ± standard deviation. Student's *t* test and chi-squared tests were used to compare the quantitative and qualitative values of the 2 groups. p values <0.05 were considered statistically significant.

Results

Table 1 and Table 2 show the demographic characteristics and side effects of treatment in the 2 groups. There was no significant difference in the age, gender, virologic responses and biochemical responses between the 2

Table 1. Demographic data for interferon-alpha (IFN-α) and IFN-α plus lamivudine treatment groups

Parameter	IFN-α alone n = 13	IFN-α plus lamivudine n = 14
Male/female	5/8	7/7
Mean age (years) [range]	37 (23-58)	41 (19-55)
Mean Knodell inflammatory score before treatment	9	8
Pretreatment alanine aminotransferase (× normal range)	2.1 (2.0-6.2)	2.4 (2.0-5.8)

groups before the start of the therapy and at the end of follow-up period (p>0.05). Although, liver biopsy specimens were taken from all patients before the start of treatment, only 2 patients in each of the groups had biopsy specimens taken after starting treatment. The pretreatment Knodell scores of the 2 patients in the combination therapy group were 9 and 8, while both of these patients had post-treatment scores of 7. The pretreatment Knodell scores of 2 patients in the monotherapy group were 9 and 7 before treatment, and were 6 and 7 post-treatment, respectively. None of the patients had an increase in serum amylase value after starting treatment. After the end of the therapy, ALT flare-up was noted in 1 patient in the combination therapy group and none of the patients in the monotherapy group. Seroconversion did not occur during follow-up in either group. In our country, the cost of 1 month of lamivudine treatment is approximately 100 US dollars. Thus, the addition of lamivudine to the regimens of these patients resulted in approximately 1200 US dollars of extra cost for 1 year.

Discussion

Chronic HBV infection is a widespread disease that affects more than 350 million people worldwide, or approximately 5% of the world's population [11]. The development of effective treatment strategies for patients with HBV remains a major clinical challenge. IFN-α and lamivudine have been approved as therapeutic agents for the treatment of chronic hepatitis B by the United States Food and Drug Administration [2]. Combination therapy with IFN-α and lamivudine has been considered to have the potential advantage of employing drugs with different mechanisms of action [12]. The first combination therapy approach used for the treatment of chronic hepatitis B was the combination of immunomodulation with viral suppression [5,7]. Investigations of combination therapy with IFN-α and lamivudine for chronic hepatitis B are limited and the reported results are controversial. Several studies concluded that combination treatment is more effective than IFN-α alone and that the effect of IFN-α might be enhanced by the lamivudine-induced inhibition of viral replication [7,13-17]. These studies reported that combination therapy had a more beneficial effect than IFN-α monotherapy in the normalization of ALT and the clearance of HBV DNA. A recent pilot study by Santantonio et al [18] demonstrated that in HBeAg-negative chronic hepatitis B, a 12-month course of lamivudine/IFN-α combination therapy was as beneficial as lamivudine monotherapy, and that the combination therapy seemed more effective in preventing or delaying the emergence of YMDD HBV variants [18]. However, the limited patient number in their study, as in the present study, does not allow definitive conclusions. Further studies including a larger number of cases are needed to determine the effects of monotherapy versus combination therapy on the risk of YMDD mutation.

Previous studies have emphasized that the efficacy of IFN-α with lamivudine was not significantly better than monotherapy [19,20]. The present study compared the biochemical and viral responses of these 2 groups before starting treatment and after treatment for 24, 52 and 72 weeks. Lamivudine was continued for 1-year in our study protocol. All of the patients in the study were treatment-naïve. We found similar

Table 2. Side effects in interferon-alpha (IFN-α) and IFN-α plus lamivudine treatment groups

Side effect	IFN-α alone n = 13	IFN-α plus lamivudine n = 14
Fever	12	13
Flu-like syndrome	11	11
Headache	6	7
Arthralgia	6	7
Depression	2	0
Nausea	4	6
Diarrhoea	2	6
Leucopenia	3	6
Flare-up of ALT after discontinuation of therapy	0	1

Abbreviation: ALT = alanine aminotransferase

results for the 2 groups at the end of the follow-up period. Our results indicate that the efficacy of combination therapy was not significantly better than IFN-α monotherapy at week 24, 52 and 72 after starting therapy.

For a therapy to be proposed as an alternative, it should be either more efficient or cheaper, or have fewer side effects. Our study suggests that the IFN-α/lamivudine combination did not have any of these superiorities. This combination therapy will invariably increase the cost of treatment. In addition, although drug-resistant mutants have not been reported after IFN-α monotherapy, a previous study found that lamivudine-resistant mutants were detected in 32% of patients treated with lamivudine at the end of a 1-year course [21]. Furthermore, 1 of the patients in the combination group in this study developed flare-up. The potential risk of ALT flare after lamivudine treatment has been documented and these flares may be fatal [22,23]. Considering these disadvantages, indications for the combined use of these agents for the treatment of chronic HBV should be reconsidered.

Our study had several limitations. First, the study groups were small and therefore it might have been more difficult to distinguish differences between them. Recent guidelines suggested that HBeAg-negative patients should be treated with IFN-α for at least for 1 year [2]. Thus, we had to terminate the present study in 2001 due to the limited number of cases that met this requirement. However, we consider this study to be of value in raising questions about the potential benefits of combination IFN-α and lamivudine treatment for patients with HBeAg-negative chronic hepatitis B. Another limitation in this study was that liver histology after treatment was available for only 4 patients. Further study of post-treatment histology involving a greater number of patients would be more informative. Another limitation of this study was that the sustained response was determined for up to 12 months post-treatment for the IFN-α monotherapy group, but at 6 months post-treatment for the combination therapy group. Thus, we could only assess the comparative efficacy of the combination therapy for up to 6 months.

In conclusion, the results of this study indicate that IFN-α monotherapy for the treatment of HBeAg-negative chronic hepatitis B patients is effective and that the addition of lamivudine does not result in superior efficacy in the treatment of HBeAg-negative patients with chronic hepatitis B. Larger, well-designed placebo-controlled studies are needed to confirm the comparative efficacy of these 2 regimens for HBeAg-negative chronic hepatitis B patients.

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