Comparison of 12-Month Courses of Interferon-α-2b–Lamivudine Combination Therapy and Interferon-α-2b Monotherapy among Patients with Untreated Chronic Hepatitis B

Kendal Yalcin,1 Halil Degertekin,1 Fetin Yildiz,3 and Yusuf Celik2

1Division of Hepatology, Department of Internal Medicine, and 2Department of Biostatistics, Dicle University School of Medicine, and 3Department of Pathology, State Hospital, Diyarbakir, Turkey

We compared the use of prolonged synchronous combination therapy with interferon (IFN)-α-2b and lamivudine with the use of IFN-α-2b monotherapy in patients with untreated hepatitis B e antigen (HBeAg)–positive chronic hepatitis B virus (HBV) infection. Thirty-three patients received therapy with lamivudine (100 mg daily) and IFN-α-2b (10 million U 3 times per week) for 12 months; 16 patients received IFN-α-2b alone (10 million U 3 times per week for 12 months). The primary end point was sustained suppression of HBV DNA and HBeAg seroconversion, which was observed in 15 (45%) of 33 patients treated with combination therapy and in 3 (19%) of 16 patients treated with monotherapy (P = .133). Both therapeutic regimens were well tolerated. Combination therapy increased the rate of sustained suppression of HBeAg and resulted in significant improvement in Knodell histologic activity index scores, compared with monotherapy. However, there was no significant difference in rates of sustained suppression between the 2 groups at the end of follow-up.

There are 2 drugs approved for the treatment of chronic hepatitis B virus (HBV) disease: IFN-α and lamivudine. Several randomized controlled trials have shown the efficacy of IFN-α in the treatment of hepatitis B e antigen (HBeAg)-positive chronic hepatitis B [1, 2]. These studies show that ∼25%–40% of the treated patients clear both the HBeAg and the HBV DNA from serum [1]. In addition, lamivudine at a dosage of 100 mg daily for 52 weeks results in suppression of HBV DNA in the majority of patients, but relapse is common. Sustained suppression of HBV DNA and HBeAg occurs in only 16% of patients [3, 4], and the degree of lamivudine resistance increases with the duration of therapy.

The pathogenesis of HBV is driven primarily by ongoing viral replication and immune-mediated destruction of hepatocytes; thus, an ideal therapy would target both viral replication and the immune response [5]. Because the 2 drugs have different mechanisms of action, the combination of the antiviral properties of lamivudine with the immunomodulatory properties of IFN-α might have possible additive effects against HBV that increase its effectiveness, compared with IFN-α monotherapy [6].

Four major studies have examined the combination of IFN-α and lamivudine for HBeAg-positive chronic hepatitis B; 2 studies were designed for previously untreated patients [7, 8], and 2 were designed for patients whose disease had previously failed to respond to IFN-α monotherapy [9, 10]. These studies found that ad-
ministration of either IFN-α in combination with lamivudine for the entire course of therapy [8] or addition of IFN-α to the regimen 8 weeks after initiating lamivudine therapy [7] resulted in a significantly higher response rate in HBeAg-positive patients. However, these studies have provided little support for the use of IFN-α-lamivudine combination therapy. Therefore, we conducted a prospective, randomized, nonblinded, and non–placebo-controlled study of a longer duration of synchronous combination therapy to investigate whether treatment prolongation could enhance the rate of sustained response in patients with HBeAg-positive chronic hepatitis B.

PATIENTS AND METHODS

Patients. All adult patients seen at the Hepatology Division, Dicle University Hospital (Diyarbakir, Turkey), both men and women, were enrolled in the study if the following criteria were met: the patient was aged 18–60 years; (2) the patient had a positive serum test result for hepatitis B surface antigen (HBsAg), and HBeAg; (3) the patient had a positive serum test result for HBV DNA by liquid hybridization or PCR; (4) the patient had a elevated serum alanine transaminase (ALT) level (>1.5–10 times greater than the upper limit of the normal [ULN]) on 3 occasions during the 6 months before enrollment; and (5) a liver biopsy specimen obtained from the patient demonstrated histologic evidence of chronic HBV infection.

Patients were excluded at screening if they had been treated previously with IFN or had received antiviral or immunosuppressive medications; if they had tested positive for antibody to hepatitis C virus, hepatitis D virus, or HIV; if they had other causes of chronic liver disease; if they drank >40 g of alcohol per day; if they had evidence of hepatocellular carcinoma; if they had decompensated liver disease (serum bilirubin level, >2.5 times greater than the ULN; prothrombin time prolonged by >3 s; serum albumin level less than the lower limit of normal; or a history of ascites, variceal hemorrhage, or hepatic encephalopathy); or if they had any contraindications specified for use of IFN. Patients also were excluded if they were pregnant; if they had a total leukocyte count of >1000 cells/mm³, a neutrophil granulocyte count of <1000 cells/mm³, a platelet count of <100,000 cells/mL, or a hemoglobin level of <10 g/dL; or if they were unable to provide informed consent.

All patients provided written informed consent before undergoing study procedures. The study was carried out in accordance with the Declaration of Helsinki and its amendments and was approved by the local ethics committee.

Study design. This study was a single-center, prospective, randomized, nonblinded, non–placebo-controlled study comparing the effectiveness of simultaneous combination therapy with lamivudine and IFN-α with the effectiveness of IFN-α monotherapy among previously untreated, HBeAg-positive patients with chronic hepatitis B. Patients eligible at screening returned for a baseline assessment within 4 weeks. At baseline, patients were prospectively randomized to receive the 2 treatment regimens at random assignment ratio of 2:1. Thirty-three patients were randomized to receive combination therapy and 17 patients were randomized to receive IFN-α monotherapy. The patients in the combination therapy group received simultaneous combination therapy with IFN-α-2b (10 million IU sc 3 times per week) plus lamivudine (100 mg once daily) for 12 months. Patients in the monotherapy group received IFN-α-2b alone, at the same dosage and for the same duration as did patients in the combination therapy group. All of the patients had at least 12 months of posttreatment follow-up after completion of therapy.

Levels of serologic markers of hepatitis B infection (HBsAg, antibody to HBsAg [anti-HBsAg], HBeAg, antibody to hepatitis B e antigen [anti-HBeAg], IgM antibody to hepatitis B core antigen [anti-HBc IgM], and HBV DNA) were determined every 2 months during the treatment period and then at 3-month intervals during follow-up. Patients were also monitored monthly during treatment, then every 3 months during the follow-up period; complete biochemical and hematologic blood tests were performed with use of conventional methods (Aerotest Autoanalyzer; Abbott Diagnostics Division). Thyroid function tests and autoantibody levels were checked at each visit.

End points. Response to therapy was defined as HBeAg seroconversion (i.e., achievement of undetectable levels of HBeAg and detectable levels of anti-HBeAg) with achievement of undetectable levels of HBV DNA in serum (as determined by PCR) and normalization of serum ALT level. Analysis of initial response was performed 6 months after the initiation of treatment, analysis of end-of-treatment response was performed at 12 months, and analysis of sustained response was performed at 24 months. The histologic response in the liver was defined as a decrease of at least 2 points in the Knodell histologic activity index (HAI) necroinflammation score [11] at 12 months, compared with the baseline score.

A relapse after a response to therapy was defined as reversion of HBeAg, detection of HBV DNA in serum by PCR, or an increase in serum ALT level to greater than the ULN (35 IU/ L). Sudden flares in the severity of HBV disease during the treatment period were defined as intermittent elevations of aminotransferase activity to >10 times greater than the ULN and more than twice the baseline value.

Histologic analysis. All patients were requested to undergo a first liver biopsy within 1 month before study entry and a second liver biopsy within the first month of follow-up so that we could compare the findings of 2 consecutive biopsies. Liver biopsy specimens were sent for histologic assessment to a single independent histopathologist who was blinded to patient identity, treatment assignment, laboratory data, and the date the
Table 1. Pretreatment characteristics of patients with chronic hepatitis B who were treated with IFN-α–lamivudine combined therapy (IFN-3TC) or IFN-α monotherapy.

<table>
<thead>
<tr>
<th>Patient characteristic</th>
<th>IFN-3TC group (n = 33)</th>
<th>IFN-α group (n = 16)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean ± SD</td>
<td>25.0 ± 8.88</td>
<td>26.2 ± 8.52</td>
<td>.586</td>
</tr>
<tr>
<td>Median (range)</td>
<td>23 (16–60)</td>
<td>24 (16–41)</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>21.85–28.15</td>
<td>21.71–30.79</td>
<td></td>
</tr>
<tr>
<td>Sex, no. male/no. female</td>
<td>22/11</td>
<td>12/4</td>
<td>.743</td>
</tr>
<tr>
<td>Weight, median kg (range)</td>
<td>66 (48–94)</td>
<td>64 (50–91)</td>
<td>.488</td>
</tr>
<tr>
<td>ALT level, U/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>163.2 ± 79.86</td>
<td>143.6 ± 54.07</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>140.0 (50.00–356)</td>
<td>153.0 (58–240)</td>
<td>.733</td>
</tr>
<tr>
<td>95% CI</td>
<td>134.9–191.5</td>
<td>114.8–172.4</td>
<td></td>
</tr>
<tr>
<td>HBV DNA load, pg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>3258 (22–6674)</td>
<td>2866 (23–5792)</td>
<td>.250</td>
</tr>
<tr>
<td>Mean log10 value ± SD</td>
<td>3.383 ± 0.438</td>
<td>3.105 ± 0.769</td>
<td></td>
</tr>
<tr>
<td>95% CI, log10 values</td>
<td>3.228–3.539</td>
<td>2.695–3.515</td>
<td></td>
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<tr>
<td>HBeAg detected, n (%)</td>
<td>33 (100)</td>
<td>16 (100)</td>
<td></td>
</tr>
<tr>
<td>Cirrhosis present, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Hepatic inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild, n (%)</td>
<td>18 (55)</td>
<td>7 (44)</td>
<td></td>
</tr>
<tr>
<td>Moderate, n (%)</td>
<td>12 (36)</td>
<td>7 (44)</td>
<td></td>
</tr>
<tr>
<td>Severe, n (%)</td>
<td>3 (9)</td>
<td>2 (13)</td>
<td></td>
</tr>
<tr>
<td>HAI score, median (range)</td>
<td>8.00 (4–14)</td>
<td>9.50 (4–13)</td>
<td>.498</td>
</tr>
<tr>
<td>Fibrosis score, median (range)</td>
<td>0 (0–3)</td>
<td>0 (0–3)</td>
<td>.933</td>
</tr>
</tbody>
</table>

NOTE. ALT, alanine aminotransferase; HAI, Knodell histologic activity index; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen.

biopsy specimen was obtained. The biopsy specimens were scored according to the original criteria of the Knodell HAI [11].

Assays. Levels of serologic markers were assessed with a qualitative microparticle EIA (Organon Teknika). HBV DNA levels in serum were measured by means of a liquid hybridization method (Digene Hybrid Capture System; Digene), with a lower limit of detection of ~2.8 × 10^5 HBV DNA copies/mL (i.e., 1 pg/mL). All samples for which hybridization assay results were negative were analyzed by PCR (Technne Cambridge), because of the relatively low detection level of liquid hybridization. The PCR assay has a lower limit of detection of 10^3–10^4 DNA copies/mL. Monitoring of serum HBV DNA levels was done with PCR, because it was thought that use of more-sensitive HBV DNA assays could further optimize response and that this is essential for an effective therapeutic strategy.

Statistical analyses. Mann-Whitney U and Wilcoxon signed-rank tests were used to compare unpaired and paired groups, respectively. Yates’ corrected χ² test and Fisher’s exact test were used to compare the presence of response between the 2 groups, and Student’s t test was used to compare percentages. The evaluation was performed with SPSS for Windows, version 11.0.0., standard version (SPSS).

RESULTS

Study population. The enrollment of patients began in June 1998. Fifty-one adult patients were screened; of these, 50 consecutive patients with detectable levels of HBsAg, HBeAg, and HBV DNA in serum at the time of screening were recruited into the study. One patient who did not have sustained detectable levels of HBV DNA and HBeAg was excluded from the study. All patients received a full 52 weeks of therapy and completed the treatment course, except 1 patient in the monotherapy arm, who was lost to follow-up in the second month of therapy and was withdrawn from the study. Although only 1 patient was excluded from the study, this study was an on-treatment analysis.

The baseline characteristics of the 2 treatment groups were similar and are summarized in table 1; the results of the study are summarized in tables 2 and 3. The median follow-up period was 26.5 months (range, 19–36 months) for the combination...
therapy group and 27 months (range, 18–36 months) for the monotherapy group.

Virologic response: HBsAg and HBsAg seroconversion and achievement of undetectable levels of HBV DNA. The percentages of initial, end-of-treatment, and sustained responses are shown in figure 1. At the end of follow-up, the proportions of patients with HBsAg seroconversion were 54% (18 of 33 patients) in the combination therapy group and 19% (3 of 16) in the monotherapy group (P = .039). No significant difference was found in the rate of HBsAg seroconversion between the 2 groups at month 6 (P = .220) and at month 12 (P = .222).

At baseline, all patients were found to have detectable serum levels of HBV DNA (i.e., levels of >10 pg/mL). Combination therapy suppressed serum levels of HBV DNA within the first month of therapy to undetectable levels (as determined by liquid hybridization) in all patients except one. In all patients in the combination therapy group, serum HBV DNA levels were detectable by PCR at months 6 and 12, except for 1 patient, whose serum HBV DNA levels were undetectable at month 6. In the monotherapy group, the proportions of patients with undetectable serum HBV DNA levels were 38% at month 6 and 56% at month 12 (P < .001 for both comparisons).

But rates of sustained suppression of serum HBV DNA levels through month 24 did not differ significantly between the 2 groups (P = .133) (table 2).

In the combination therapy group, all relapses occurred during the first 3 months of follow-up, except 1 relapse that occurred at month 6. In all the patients in the combination therapy group who experienced relapse, serum HBV DNA levels returned to and exceeded baseline levels within 3 months after the end of treatment. This occurrence was seen in a minority of patients in the monotherapy group. Despite achievement of HBsAg seroconversion, 3 patients in the combination therapy group later had detectable levels of HBV DNA and high levels of ALT, which persisted during the entire period of follow-up. No other breakthrough biochemical and/or virological responses were seen in the combination therapy group during the course of therapy. A breakthrough viral response was seen in only 1 patient in the monotherapy group, at month 11.

Achievement of undetectable levels of HBsAg and the detectable levels of anti-HBsAg was observed in 2 (6%) of 31 patients at month 5 and month 7 in the combination therapy group, and was not observed in the monotherapy group.

Histologic response. Pretreatment liver biopsy specimens were available for study for all patients in both groups. Two patients in the combination therapy group and 1 in the monotherapy group refused to undergo a second liver biopsy. The rates of histologic response (as defined on the basis of the Knodell HAI score) were 84% (26 of 31) among patients receiving combination therapy and 27% (4 of 15) among patients receiving IFN-α monotherapy (P < .001). None of the patients in the combination therapy group had HAI scores that indicated a worsening of liver disease, whereas 1 (7%) of 15 patients receiving IFN-α monotherapy did. Moreover, significant reductions were found in the mean and median HAI necroinflammation scores between both groups (table 3). Fibrosis scores (of the HAI index) remained unchanged in each of the groups, and no significant difference between the 2 groups was found.

Biochemical response. All patients had elevated ALT levels...
Figure 1. Response rates among patients with chronic hepatitis B treated with IFN-α monotherapy or IFN-α–lamivudine combined therapy. The significance of the difference in responses between the 2 groups is indicated above the black bars. All responses are defined as normalization of serum alanine transaminase level, achievement of a serum hepatitis B virus DNA level that is undetectable by PCR, achievement of undetectable levels of hepatitis B e (HBe) antigen, and achievement of detectable levels of anti-HBe. Time points indicated are 6 months after the start of therapy; month 12 corresponded to the end of therapy, and month 24 was 12 months after the end of therapy.

at baseline. During the 12 months of therapy, serum ALT levels returned to normal and remained so in 28 (85%) of 33 IFN-α–lamivudine recipients and 11 (69%) of 16 IFN-α recipients ($P = .351$). At least 12 months after therapy, 48% of patients in the combination therapy group (16 of 33) had a sustained ALT response, as compared with 19% of the patients in the monotherapy group (3 of 16; $P = .060$). During treatment, the incidence of sudden increases in the ALT level was 31% (5 of 16) in the patients who received IFN-α alone and 30% (10 of 33) of those who received the combination therapy. All such increases occurred during the first 3 months of therapy and resolved spontaneously without any intervention.

**Safety.** In this study, no serious adverse events were observed in either group. The incidence of adverse events was similar in both treatment groups. The most common adverse events during treatment were malaise (in 73% of patients), hair loss (in 64%), myalgia (in 61%), fever (in 50%), weight loss (in 50%), and anorexia (in 47%). There was a high incidence of mouth dryness in the combination therapy group, compared with the monotherapy group (25 of 33 vs. 3 of 16, respectively; $P < .001$). Hyper- or hypothyroidism and elevations of serum amylase or creatinine phosphokinase levels were not detected in any patient. No patient required dose reduction or interruption of therapy, and the severity of symptoms decreased after a few weeks. The good compliance with therapy observed in this study may reflect the high motivation of patients. There were no deaths or instances of hepatic decompensation during the study.

**DISCUSSION**

Until now, the short-term use of IFN-α alone or in combination with lamivudine has not been reported to be effective for treatment of chronic hepatitis B infection. Data on combination therapy are few and are restricted to studies of courses of treatment of 4–6 months’ duration. Moreover, the 4 published trials of combination therapy provide little support for the use of the IFN-α–lamivudine combination. Also, the design of the initial studies of combination therapy may not have been optimal for showing the full effects of combination therapy [5, 12, 13]. On the other hand, in many controlled trials, it has been shown that there was an increased clearance of both HBeAg and HBV DNA after prolonged IFN-α therapy, compared with treatment for the standard period of 16 weeks [14, 15].

Our study demonstrates that administration of combination therapy for 12 months to HBeAg-positive patients induces rapid inhibition of viral replication, normalization of liver function, and histologic evidence of improvement in liver disease. The study found a high rate of HBeAg seroconversion (54% of patients) after 12 months of combination therapy, a finding that is not in accordance with the seroconversion rate of 29% after 16 weeks of combination therapy reported by Schalm et al. [7], nor with the seroconversion rate of 33% after 24 weeks of therapy reported by Barbaro et al. [8]. The enhanced efficacy of prolonged treatment was so pronounced that, in future treatment regimens that include combination therapy, prolongation of therapy for up to 52 weeks should be considered.
Despite a relatively high mean baseline ALT levels (≤4.0 times greater than the ULN) and a long course of therapy, the low rate of HBeAg seroconversion among the patients who received IFN-α monotherapy seems to be conflicting and discordant with the findings of other studies: one study reported a seroconversion rate of 15% among patients with a low mean baseline ALT [16] and another reported a seroconversion rate of 37% among patients with a relative high mean baseline ALT levels [17]. This may be the result of high virus loads or to an early acquisitions of infection in the study patients; both of these conditions are associated with a poor response to IFN-α [18]. However, given a rather large confidence interval, due to the low number of patients enrolled, the 19% seroconversion rate is not surprising.

The pretreatment level of viral replication in serum, as indicated by the quantitative HBV DNA values, has been implicated as a strong response-predictive variable in many studies of IFN-α therapy for chronic hepatitis B [17–19]. By reducing the HBV DNA concentration, lamivudine may help to overcome cytotoxic T lymphocyte hyporesponsiveness and restore T cell reactivity against HBV [20–22] and may have effect on improving the efficacy of IFN-α. It is theoretically possible that the use of the nucleoside analogues first may interrupt cytotoxic T lymphocyte activation by means of interference with viral transcription and appropriate viral peptid expression on the surface membranes of hepatocytes. In other words, providing the IFN first, in a lead-in phase of treatment, may be a viable option.

Unfortunately, despite the impressive HBeAg seroconversion rates achieved at month 24, the difference in sustained response rates between the 2 groups remained nonsignificant at that time. Viral persistence was observed after anti-HBeAg seroconversion in 3 patients in the combination therapy group, and this influenced the level of significance. This might be explained by the presence of a mixed virus population with complete suppression of the wild-type virus after cessation of therapy with IFN-α and lamivudine combination therapy, or by persistence or reemergence of a mutation in the precore region. It has been shown that viral persistence after anti-HBeAg seroconversion that was induced by IFN or lamivudine therapy is often associated with the presence of circulating HBV genomes harboring mutations in the precore promoter region [23]. Thus, a precise monitoring of viremia levels with more-sensitive assays and monitoring for the presence of HBV mutant strains is warranted for patients undergoing antiviral therapy, and the sustained response to therapy should be the most clinically relevant result used in therapeutic trials.

Histologic evidence of improvement in liver disease is a significant marker of biologic improvement after therapy, especially when the number of patients is low. In the present study, combination therapy significantly reversed necroinflammatory activity, compared with IFN-α monotherapy. This large benefit occurred regardless of patients’ HBeAg seroconversion status and treatment response status in the combination therapy group. The 84% rate of improvement in hepatic inflammation observed in our study patients was higher than the rate of 46% reported by Barbaro et al. [8], the rate of 56% after 1 year of lamivudine therapy reported by Lai et al. [4], and the rate of 54% after a 24-month course of IFN-α therapy reported by Lampertico et al. [24]. Adding a prolonged course of IFN-α therapy to a course of lamivudine therapy seems to double the substantial beneficial effect of lamivudine on liver disease.

The main limitation of this study is the small number of patients; this makes it difficult to draw precise conclusions, because it is difficult to compare the results obtained with a small number of patients with the results of much larger studies. Other limitations include the observed pattern of fibrosis, which may not be well distributed and may have influenced the results positively in both groups; and the very young age of the patients, which is also a limitation regarding this study’s generalization. Sequencing of the HBV genome for tyrosine-methionine-aspartate-aspartate (YMDD) mutations was not performed. Thus, the effect of YMDD mutations, if there were any, on the patients’ response remained undefined. But it is important to note that there was no viral or biochemical breakthrough in any patient during therapy in the combination therapy group.

In the present study, both treatment regimens were safe and well tolerated, and the efficacy of concomitant IFN-α and lamivudine therapy appears to be better than that of IFN-α monotherapy and the previously studied sequential administration of IFN and lamivudine. Several factors may possibly explain this difference: (1) a synchronous combination regimen is superior to a sequential combination regimen; (2) a prolonged course of combination therapy is more effective than shorter course of combination therapy; (3) combination therapy is better than monotherapy; and (4) patients enrolled in this study tended to have a higher mean ALT levels, a higher HAI score, and a lower stage of fibrosis. We conclude that IFN-α and lamivudine combination therapy, in combination with a longer duration of therapy, appears to be a therapeutic regimen and might be considered for the initial treatment of patients with chronic hepatitis B.

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References


