Pegylated Interferon Alpha-2b as Monotherapy or in Combination With Ribavirin in Chronic Hepatitis Delta

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Therapy of chronic hepatitis delta with standard interferon therapy has met with limited efficacy. This study was designed to examine the efficacy and safety of peginterferon with or without ribavirin. Thirty-eight serum hepatitis B surface antigen- and HDV RNA-positive patients with alanine aminotransferase (ALT) more than 1.5 times the upper normal limit received peginterferon alpha-2b (1.5 μg/kg) alone as monotherapy (n = 16) or in combination with ribavirin (n = 22), for 48 weeks. Thereafter, all the patients were maintained on peginterferon for 24 weeks and followed for 24 weeks off therapy. The primary end point studied was the virological and biochemical response at the end of follow-up. HDV RNA was determined by single or nested polymerase chain reaction assays. Twenty-seven patients (71%), 11 receiving monotherapy and 16 receiving the combination treatment, completed the follow-up. At the end of treatment, a virological response was observed in 3 of the patients treated with peginterferon (19%) and in 2 of the patients treated with combination therapy (9%), and a biochemical response was observed in 6 (37.5%) and 9 patients (41%), respectively. In nonresponders, ALT diminished from a mean of 174 ± 53 to 86 ± 41 IU/L. At the end of follow-up, serum HDV RNA was negative in 8 patients (21%), and a biochemical response was detected in 10 patients (26%). Treatment was discontinued in 25% of the patients, and dosing was modified in 58%. In conclusion, a prolonged course of peginterferon alpha-2b resulted in clearance of serum HDV RNA and ALT normalization in a fifth of patients with chronic hepatitis D, while ribavirin had no effect on the viral clearance rate. Overall tolerance of therapy was poor. (HEPATOLOGY 2006;44:713-720.)

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Hepatitis delta virus (HDV) infection has a broad spectrum of clinical manifestations but generally develops into a chronic, severe disease.¹ Though the last 2 decades have seen a substantial decline in the circulation of HDV and the incidence of new forms of the disease in southern Europe,²,³ there is still residual manifestation of the disease in patients infected years ago when HDV was endemic; these patients pose a difficult therapeutic challenge, as most have advanced disease and cirrhosis.

At present, interferon alpha is the only option for treating chronic hepatitis D; however, the response is limited and variable depending on the different schedules of treatment.⁴⁻⁶ Up to 70% of patients may reach a normal aminotransferase level while on therapy, but the relapse rate is high after discontinuation. Therapeutic efficacy increases when higher doses of interferon alpha (9 MU t.i.w.) are administered for prolonged periods (12-24 months).³,⁴ Twelve years of treatment with a high dose of interferon alpha achieved complete resolution of the infection in a single HDV patient, with remission of liver fibrosis and disappearance of the HDV and hepatitis B virus (HBV) from serum.⁷ Improvement in clinical outcome and survival has been reported even in patients with cirrhosis at baseline.⁸ Unfortunately, compliance with an intensive
interferon (IFN) regimen has been poor because of the dose-dependent development of side effects.9 Other anti-viral agents, whether given as monotherapy or in combination, have not proven to be beneficial.10-12 Ribavirin (RBV), a guanosine analogue, was able to inhibit HDV replication in primary woodchuck hepatocyte cultures infected with HDV; its mechanism of action against HDV is not fully understood, although the mechanism may involve inhibition of viral mRNA formation. However, the in vitro results were not reproduced in vivo in a pilot study of 9 patients with chronic hepatitis D given RBV monotherapy.14 In a recent study, the association of RBV with interferon alpha-2a in chronic hepatitis delta was found to provide no additional benefit than treatment with interferon alone.15

Pegylated IFN (PEG-IFN), the product of conjugation of interferon with poly(ethylene glycol), could be more effective than conventional interferon because of its improved pharmacodynamics and pharmacokinetics. By prolonging the plasma half-life and providing protracted activity of the cytokine, PEG-IFN allows for once-weekly dosing and stable therapeutic levels. The new formulation of long-acting interferon is also likely to provide better compliance if the drug has to be administered for a protracted time, as is required in chronic hepatitis D. The current study was designed to compare the efficacy and safety of PEG-IFN alpha-2b monotherapy given for 72 weeks versus therapy with a combination of PEG-IFN alpha-2b with RBV given for 48 weeks, followed by PEG-IFN monotherapy for 24 additional weeks.

**Patients and Methods**

**Patients.** Thirty-eight patients with chronic hepatitis delta who had been referred to 3 Italian medical centers (in Torino, San Giovanni Rotondo, and Naples) were enrolled. Inclusion criteria were the presence of antibodies to hepatitis delta antigen (anti-HDV) and hepatitis B surface antigen (HBsAg) in serum for at least 6 months prior to screening, detectable serum HDV RNA at enrollment, and alanine aminotransferase (ALT) ≥ 1.5 and ≤ 10 times the normal value. Patients with compensated cirrhosis were eligible if their hemoglobin was ≥ 12 g/dL, their WBC > 3,000/mm³, and their platelets > 80,000/mm³. Patients were excluded if they were hepatitis C virus (HCV) or HIV positive, had received antiviral, cytotoxic, corticosteroid, or immunomodulatory treatment within 6 months of inclusion, or had a history of psychiatric disorders. Hospital ethics committees approved the study; written informed consent was obtained from all patients prior to enrollment.

The patients were randomly assigned on the basis of a computer-generated list to one arm of a 2-arm study: in one arm, patients were given PEG-IFN alpha-2b (1.5 μg/kg of body weight subcutaneously weekly) and RBV (800 mg orally daily) for 48 weeks followed by PEG-IFN alpha-2b monotherapy for 24 additional weeks; in the other arm, patients were treated with PEG-IFN alpha-2b monotherapy for 72 weeks at the same dosage. After completing treatment, all patients were followed up for 24 weeks.

**Laboratory and Virological Testing.** Assessment of the efficacy and safety of the treatments was carried out at baseline, at weeks 2, 4, 6, 8, and 12, and then every 6 weeks until week 72. Off treatment, follow-up assessments were carried out at 4, 12, and 24 weeks. At each clinical visit, routine laboratory tests performed included serum ALT and aspartate aminotransferase, serum direct and total bilirubin, albumin, and complete blood counts. Serologic markers of HBV, HDV, HCV, and HIV were tested by commercial enzyme immunoassays (Abbott Laboratories, North Chicago, IL; Sorin Biomedica, Saluggia, Italy; Ortho Diagnostic Systems, Raritan, NJ). IgM anti-HD was measured using a previously described capture immunoassay.16 Serum samples were tested for HDV RNA as previously reported17 by semiquantitative single and nested polymerase chain reaction (PCR) assays, with a sensitivity of approximately 1,000 and 1-10 genomes/mL respectively. Serum HBV DNA levels were determined with a commercial quantitative PCR assay (Ampligic HBV Monitor Test, Roche Diagnostics, GmbH Mannheim, Germany) with a sensitivity threshold of about 400 copies/mL.

**Liver Histology.** A liver biopsy was performed within 24 months prior to screening and at the end of treatment. A single pathologist, blinded to study treatment, assessed all biopsies. Changes in histological activity were evaluated by the modified Ishak score.18

**End Points.** The primary efficacy end point was the ability of PEG-IFN, as monotherapy or in combination with RBV, to clear HDV viremia or to suppress HDV RNA levels below 1,000 copies/mL. Secondary efficacy objectives included biochemical response, improvement of histological score in paired pre- and post-treatment liver biopsies and loss of HBsAg with seroconversion to antibodies to hepatitis B surface antigen (anti-HBs). As in previous investigations5,6 the virological response was defined as the loss of serum HDV RNA at the end of treatment (end-of-therapy virological response) or during the 6-month post-therapy follow-up period (sustained virological response). Relapse was defined as the reappearance of serum HDV RNA after treatment. A biochemical response was defined as a normal serum alanine aminotrans-
ferase level at the end of treatment (end-of-therapy biochemical response) or after 6 months of follow-up (sustained biochemical response). A relapse was defined as an increase in the serum alanine aminotransferase level to more than 1.5 times the upper limit of normal after a biochemical response.

Statistical Analysis. The SPSS statistical package (version 6.1.3; SPSS Inc., Chicago, IL) was used to analyze the data. Continuous variables are expressed as means and standard deviations (SDs). Categorical variables are reported as percentages. Baseline characteristics of the 2 treatment groups were compared with the Student t test if continuous variables or the $\chi^2$ test (or Fisher’s exact test, when needed) if categorical variables. A $P$ value of < .05 was considered statistically significant. Predictors of response to therapy were analyzed by univariate and multivariate analyses. The intention-to-treat approach was taken by including all patients and per protocol analysis at 24, 48, and 72 weeks by including all patients who completed treatment.

Results
Sixteen patients were randomized to PEG-IFN alpha-2b monotherapy, 22 to treatment with PEG-IFN alpha-2b in combination with RBV. The baseline clinical, demographic, and histological features of patients are given in Table 1: the patients in the 2 treatment arms had similar features at baseline. Thirty patients had previously received treatment, 17 with interferon, 2 with lamivudine, and 11 with both lamivudine and interferon. Of the 28 patients treated with IFN, 25 patients had previously received a 48-week course of interferon alpha-2a, 5 of whom received a dose of 9 million units 3 times a week and 20 of whom received 5 million units. Intolerance caused the remaining 3 patients to stop therapy after 3 months of treatment with interferon alpha at a dose of 5 million units 3 times a week. By the end of their previous treatments, 19 patients had not responded, 7 patients had normalized serum ALT levels but remained viremic, and 2 patients exhibited both biochemical and virological responses. A virological relapse occurred in both responders 1 to 10 months after cessation of therapy.

Twenty-eight of the 38 patients had cirrhosis; 12 of these patients were assigned to receive the monotherapy (75%) and 16 to receive the combination therapy (73%).

Eleven of the 38 patients (29%) did not complete treatment, 5 (31%) in the monotherapy group and 6 (27%) in the combination group; they dropped out of the study after an average of 29 weeks (range 4-54 weeks; Fig 1). Nine of the 11 patients stopped treatment because of poor compliance or for personal reasons; the remaining 2 ceased treatment because of adverse events, which is discussed in the Safety section. Because this was an intention-to-treat analysis, dropouts were included as nonresponders in therapy outcomes.

Biochemical Response. By the end of treatment a biochemical response was achieved in 15 patients (39%), 6 who had been in the monotherapy group (37%) and 9 in the combination therapy group (41%); the difference was not significant ($P = .83$). In the 23 nonresponders, mean ALT level diminished from 174 ± 110 IU/L (range 74-185) to 86 ± 61 IU/L (range 49-184). At the end of follow-up, the biochemical response was maintained in 6 of the 15 patients with end-of-therapy response (3 in each treatment arm), whereas the remaining 9 patients relapsed, 3 who had received the monotherapy and 6 who had received the combination therapy. In the 9 relapers, ALT level started to increase again 1-3 months after stopping therapy; in 3 patients aminotransferase showed as much as 5- to 10-fold increase above the upper normal limit, an elevation that persisted up to the 16th month.
of follow-up. In 4 nonresponders ALT values dropped to normal during follow-up. At the end of follow-up, 10 patients had normal ALT values.

The pattern of ALT fluctuation during and off therapy is shown in Fig. 2. During therapy ALT was higher than baseline in 9 patients (7 of them treated with combination therapy) and reached peak values by week 24; in 2 patients the enzyme levels were more than 10-fold higher than the upper normal limit. Hepatic flares were not associated with jaundice or symptoms of hepatic decompensation. Because of a
concomitant increase in gammaglutamyltransferase, treatment was discontinued in 1 patient. No association was found by univariate analysis between biochemical response with treatment regimen, duration of disease, age, sex, risk factors for HDV transmission (familial history, blood transfusion, drug addiction), histology, body weight, or previous therapy with lamivudine and/or interferon.

**Virological Response.** After 72 weeks of therapy, serum HDV RNA was undetectable in 5 patients (13%), 3 of them in the monotherapy arm. More patients who received peginterferon alpha-2b monotherapy achieved an end-of-therapy response than did those who received the combination therapy (19% vs. 9%), but the difference did not reach statistically significance because of the small sample size. In 8 other patients (21%) viremia diminished to less than 1,000 copies/mL by the end of treatment; 3 had received monotherapy (19%) and 5 had received the combination regimen (23%); this difference was not significant \( (P = 1) \). Altogether, 13 patients had undetectable or very low HDV RNA levels at the end of therapy (Table 2).

A sustained virological response was obtained in 5 patients who showed end-of-treatment response; an additional 3 patients whose tested HDV RNA level was less than 1,000 copies/mL at the end of therapy had undetectable HDV RNA at the end of follow-up. The rates of patients with negative, low (<1,000 copies/mL), and high (≥1,000 copies/mL) viremia levels during treatment and at the end of follow-up are shown in Fig. 3. At the end of follow-up, 8 patients (21%) were HDV RNA negative, 4 in each treatment arm. The virological response was 88.2% in patients with no risk factor versus 11.8% in patients with risk factors for viral transmission \( (P = .009; \ OR = 4.5; \ 95\% \ CI = 1.1-17.6) \). The 87.5% rate of virological response in previously untreated patients was significantly higher than the 35.7% rate in patients previously treated with standard interferon and/or lamivudine \( (P = .09; \ OR = 1.6; \ 95\% \ CI = 1.1-2.4) \). In the multivariate analysis, not having risk factors for viral transmission and being treatment naive were independent predictors of a sustained virological response. There was no significant association between HDV viremia variations with treatment regimen, duration of disease, age, sex, liver histology, or body weight.

**Anti-HDV Antibodies and HBV Markers.** All patients were positive for IgM antibodies to hepatitis delta

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### Table 2. Rates of Biochemical and Virological Responses in Patients Treated With Peginterferon Alpha-2b as Monotherapy or in Combination With Ribavirin

<table>
<thead>
<tr>
<th>Response at End of Therapy</th>
<th>Response at End of Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Patients (N = 38)</td>
<td>PEG-IFNAlpha-2b (N = 16) + RBV (N = 22)</td>
</tr>
<tr>
<td>Normal ALT (N %)</td>
<td>15 (39%)</td>
</tr>
<tr>
<td>Virological response</td>
<td></td>
</tr>
<tr>
<td>HDV RNA ≤ 1,000 cp/mL</td>
<td>8 (21%)</td>
</tr>
<tr>
<td>HDV RNA -ve</td>
<td>5 (13%)</td>
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| Biochemical response       | 10 (26%)                    | 4 (25%)                    |
| Virological response       | 4 (10%)                     | 1 (6%)                     |
| HDV RNA -ve                | 8 (21%)                     | 4 (25%)                    |

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Fig. 3. HDV RNA levels during therapy and after 24 weeks of follow-up in patients treated with PEG-IFN or PEG-IFN plus RBV.
antigen (IgM anti-HD) at baseline, with a mean titer of $10^{-4}$. The IgM titer remained unchanged in nonresponder patients, in biochemical but not virological responders, and in those who experienced a decrease in HDV RNA levels on therapy. In the 5 virological responders, the mean IgM anti-HD titer decreased by 1 log. Anti-HD IgM antibodies disappeared in 2 sustained responders after they had been off therapy for 6 months.

During follow-up no patient lost HBsAg or seroconverted to anti-HBs. All but one patient were positive for antibodies to hepatitis B e antigen at baseline and throughout the study. Two of the 7 patients with a baseline HBV DNA level higher than 1,000 copies/mL had levels of at least $10^5$ copies/mL: in both patients HBV DNA viremia declined from baseline but rebounded when treatment was stopped.

Liver Histology. Only 6 patients, 3 with an end-of-therapy biochemical response, 1 with a sustained biochemical and virological response, and 2 nonresponders, consented to a second biopsy at the end of the 72 weeks of therapy. No worsening was documented in paired pre- and post-treatment biopsy specimens. Necroinflammatory activity improved in 2 patients (≥2 points), fibrosis in 1 patient, and both in another patient (Table 3). Histology also improved in 2 patients who did not achieve a biochemical or virological response.

Safety. Two patients, one of whom was with treated with the combination treatment, discontinued therapy for safety reasons. In one patient ALT increased at week 24 in association with an increase in gammaglutamyltransferase to a level 8.5 times the upper normal limit. The other patient withdrew at week 4 because of being in a prolonged lethargic state, secondary to the assumption of antidepressants. Nine patients, 4 of them treated with monotherapy and 5 with combination therapy, withdrew consent while on therapy.

Adverse events were typical of interferon alpha treatment. They included fatigue (n = 14), headache (n = 12), insomnia and irritability (n = 11), arthralgia (n = 9), cellulitis, urinary and respiratory infections (n = 8), generalized itching (n = 6), nausea (n = 5), injection site reactions (n = 2), and depression (n = 1). Itching prevailed in patients treated with RBV; the other side effects were similarly distributed in the 2 treatment arms. Dose modifications of PEG-IFN and/or RBV were required for 22 patients (58%), 8 (50%) of whom were treated with PEG-IFN monotherapy and 14 (63%) of whom received combination therapy ($P = .6$). Reasons for dose modification were thrombocytopenia (n = 13 patients, 6 treated with PEG-IFN and 7 with combination therapy), neutropenia (n = 8 patients, 2 treated with monotherapy and 6 with PEG-IFN and RBV), anemia (n = 4 cases, all treated with PEG-IFN and RBV), and gammaglutamyltransferase elevation (in a single patient treated with combination therapy).

Discussion

Hepatitis D virus infection has considerably diminished in southern Italy in the last 2 decades; in Italy, the prevalence of anti-HD in chronic HBsAg carriers with chronic liver disease has declined from 25% at the beginning of 1985$^2$ to less than 10% in recent years.$^9$ Despite the diminished epidemiological burden, the clinical impact of residual disease in the cohorts of patients infected when HDV was endemic remains appalling, as there as yet no efficacious therapy for hepatitis D, and for most patients the disease has now progressed to cirrhosis.$^2$ The present study, one of the first to evaluate the efficacy and tolerability of treatment with PEG-IFN alpha-2b with or without RBV in these difficult-to-treat patients, has shown that a prolonged, 72-week course of PEG-IFN induced the sustained clearance of HDV in a fifth of those treated. There was also decreased necroinflammation in 2 of 4 patients for whom paired biopsies were available; however, this number was too limited to draw conclusions on the efficacy of PEG-IFN on liver histology.

Consistent with the results of a recent study,$^{15}$ adding RBV to IFN did not result in greater benefit for any biochemical, virological, or histological end points considered. These results were achieved at the expense of relatively important side effects; treatment needed to be interrupted in 29% of the patients, mostly because of poor compliance. Two patients had a severe flare of hepatitis while on a full dose of PEG-IFN alpha-2b, both during week 2 of therapy; fortunately the increase in aminotransferase levels was transient and not accompanied by symptoms of hepatic decompensation or cholestasis. Hepatic flares also were reported during standard interferon alpha treatment.$^{19,20}$ In contrast to the frequent and severe psychiatric side effects observed during standard interferon therapy,$^9,21$ our patients reported insomnia,
irritability, and depression of moderate intensity and usually did not require pharmacological support.

Of the 38 patients evaluated, 28 had a histological cirrhosis, and 30 had undergone previous courses of standard IFN therapy; both factors diminished the response to IFN in both HCV- and HBV-related liver disease\(^{22,24}\) and possibly explained, at least in part, the relatively low rate of HDV clearance achieved by our patients. Naive patients without cirrhosis might respond better\(^{25}\); in the post hoc analysis of the 8 patients in this study who were naïve for previous therapy, HDV infection cleared in 3 cases (37.5%). In a recent study of 31 patients with chronic hepatitis D, including many (75%) without cirrhosis, 20% cleared HDV after 2 years of treatment with standard IFN.\(^1\)

Consistent with the superior results achieved in therapy of chronic HBV and HCV diseases using pegylated rather than standard IFN, the results of our study would also support the use of PEG-IFN as a first-line therapy and the recommendation to initiate therapy as early as possible for those with chronic hepatitis D. In fact, the efficacy of standard IFN-α in the treatment of chronic delta hepatitis (CHD) remains controversial; the few controlled and the many small uncontrolled trials of the use of standard IFN as a treatment for CHD found that HDV synthesis was suppressed in a fraction of patients while on therapy, but treatment failed to induce sustained clearance of the virus post-therapy; transient and occasionally even permanent clearance of HDV can also occur spontaneously in untreated patients.\(^{26}\) At variance with the delayed clearance of HBsAg after spontaneous or standard IFN-induced clearance of HDV RNA from serum,\(^{21-27}\) in the present study none of the patients treated with PEG-IFN who achieved a sustained response cleared this antigen, possibly because of the relatively short post-treatment follow-up.

Of note, the administration of PEG-IFN for 72 weeks made HDV undetectable in 5 patients while on therapy, but 3 patients cleared HDV only after therapy was discontinued. A similar pattern of delayed response has been observed in chronic hepatitis D also after discontinuation of conventional interferon.\(^8\)

In conclusion, our study has provided evidence that a 72-week course of therapy with PEG-IFN alpha-2b may result in sustained clearance of HDV in about a fifth of patients with chronic hepatitis D, even though most of our patients had cirrhosis and had been unresponsive to a previous course of monotherapy with standard IFN.

Acknowledgment: The authors thank Schering-Plough for providing PEG-IFN alpha-2b and ribavirin.

References


