Efficacy of Peginterferon alpha-2b in Chronic Hepatitis delta: Relevance of Quantitative RT-PCR for Follow-Up

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Hepatitis delta virus (HDV) can cause severe acute and chronic liver disease in patients infected by hepatitis B virus. Interferon alpha at high doses, although poorly efficient, is the only treatment reported to provide some benefit in chronic hepatitis delta. Pegylated interferon alpha (PEG-IFN) has not yet been evaluated. Treatment is usually monitored by the qualitative detection of HDV-RNA in serum. In this study, safety and efficacy of PEG-IFN were assessed in chronic hepatitis delta, and serum HDV-RNA kinetics were determined using quantitative RT-PCR. Fourteen patients with chronic hepatitis delta received subcutaneous PEG-IFN alpha-2b during 12 months (1.5 µg/kg per week). Serum HDV-RNA was quantified at initiation and during the course of therapy, and during the posttreatment follow-up period, which ranged from 6 to 42 months (median 16 months). PEG-IFN alpha-2b was well tolerated, inducing no serious adverse effect. Sustained biochemical response was obtained in 8 patients (57%). At the end of treatment, 8 patients (57%) had achieved virological response (undetectable HDV-RNA). Sustained virological response throughout the posttreatment follow-up period was observed in 6 patients (43%). HDV-RNA kinetics were predictive of the response: after 3 months of PEG-IFN, HDV-RNA levels were significantly lower in the responders than in the nonresponders group (P = .018). After 6 months of therapy, a negative HDV-RNA was predictive of sustained response (P = .021). In conclusion, this preliminary study indicates that PEG-IFN alpha-2b is safe and efficient for treatment of chronic hepatitis delta. The follow-up of HDV-RNA levels during therapy, which allows the differentiation of various profiles of virological responses, improves treatment monitoring. (HEPATOLOGY 2006;44:728-735.)

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Hepatitis delta virus (HDV) is a satellite RNA virus, which depends on the hepatitis B virus (HBV) for virion assembly and propagation.1 HDV infection can cause severe acute and chronic liver disease, with a chronicity rate reaching 70% to 90% in cases of super-infection,1-3 and a common progression to cirrhosis (60%-70%) accounting for frequent evolution to end-stage liver disease and hepatocellular carcinoma.4,5 To date, treatment of chronic hepatitis delta relies on interferon alpha (IFN), but the efficacy is limited.6 Antiviral drugs such as ribavirin, acyclovir, or famcyclovir are inefficient,7,8 and anti-HBV drugs such as lamivudine, which efficiently reduce HBV viremia but not HBsAg levels have no effect on hepatitis delta,9 even when associated to IFN.10 Clevudine, which induces a reduction in the covalently closed circular DNA replication template levels could be useful in the treatment of chronic hepatitis delta but has only been evaluated in the woodchuck model for this indication.11 The pegylated form of IFN (PEG-IFN), which has been proved to be more efficient than standard IFN in chronic hepatitis B and C, might improve the outcome of chronic hepatitis delta, but such treatment has not yet been evaluated.12,13

The diagnosis of HDV infection usually relies on the detection of specific anti-HDV antibodies, with the presence of anti-HDV IgM reflecting ongoing viral replication. However, serological approach for the detection of virus replication lacks sensitivity and HDV-RNA detec-
tion in serum appears to be the most suitable means to assess active HDV infection. As for other chronic viral infections, quantification of HDV-RNA levels in serum might improve the monitoring of treatment efficacy, but the relevance of viral load determination in the management of hepatitis delta remains to be established. However, HDV-RNA quantification might help with the understanding of the physiopathology of HDV infection, contribute to the evaluation of the severity of the liver disease and improve treatment monitoring.

The aims of our study were (1) to evaluate the efficacy and safety of PEG-IFN alpha-2b therapy in patients with chronic hepatitis delta and (2) to assess the usefulness of HDV-RNA quantification to follow the response to treatment. For this purpose, fourteen patients with chronic hepatitis delta received PEG-IFN alpha-2b during 12 months. Treatment efficacy and HDV-RNA kinetics were evaluated during the course of the treatment and the posttreatment follow-up.

**Patients and Methods**

**Patients.** Patients with chronic hepatitis delta, aged 28 to 52 years, were included in the study between December 2000 and December 2003. Chronic hepatitis delta was defined by the following criteria over a period of 6 months: histological evidence of chronic hepatitis, serum alanine aminotransferase (ALT) levels >1.3 fold the upper limit of normal (normal range 5 to 40 IU/L), detectable hepatitis B virus surface antigen (HBsAg), HDV total antibody and HDV-RNA in serum. Patients with another cause of chronic liver disease were excluded, as well as patients with HIV infection, serious medical illness, hepatocellular carcinoma, decompensated liver disease, platelet count <50,000/mm³ and pregnant or breastfeeding women.

**Treatment Schedule.** All patients received 1.5 μg/kg subcutaneous PEG-IFN alpha-2b (Viraferon Peg, Schering Plough) weekly for 12 months.

**Assessment of Treatment Efficacy.** Treatment was monitored with biochemical, histological and virological markers, namely serum ALT levels, METAVIR scoring of the necroinflammatory activity and fibrosis of the liver, anti-HDV IgM and HDV-RNA. Biochemical and virological responses were evaluated every 3 months during the course of the treatment (M3, M6, M9 and M12), at the end of therapy, and every 3 months during the posttreatment follow-up period. The primary efficacy parameter of the study was virological response, which was assessed at the end of therapy by undetectable HDV-RNA in the serum. Sustained virological response was defined by undetectable HDV-RNA in the serum 6 months after the end of therapy and throughout the posttreatment follow-up period. Patients were considered nonresponders when HDV-RNA was still detected in serum at the end of therapy. Biochemical response was assessed at the end of therapy by normal ALT levels.

Liver biopsies were performed before initiation of therapy in all patients, and at the end of therapy for some of them. Each biopsy was anonymously re-analyzed by a single pathologist.

**Virological Assays.** Virological assays were performed at inclusion, during the course of therapy and during the posttreatment follow-up period. HBsAg and HBeAg, as well as anti-HBs and anti-HBe antibodies were detected using the AxSYM system (ABBOTT, Rungis, France). HBV-DNA was quantified by quantitative PCR, with COBAS TaqMan HBV (Roche Diagnostics Systems, Meylan, France), sensitivity threshold, 50 copies/mL.

Anti-HCV antibodies were detected using the AxSYM HCV system V3.0 (ABBOTT). Serum HCV-RNA was detected by the use of the VERSANT HCV-RNA Qualitative Assay (TMA) (Bayer HealthCare, Puteaux, France), sensitivity threshold, 50 copies/mL.

Total antibodies to HDV were detected with ETI-AB-DELTA-2 (DiaSorin S. A, Antony, France), and specific anti-HDV IgM were detected with ETI-DELTA-IGMK-2 (DiaSorin). In this ELISA-format test, the presence or absence of IgM is determined by comparing the absorbance value of the unknown sample to that of the cutoff value. Samples with absorbance values repeatedly within ±10% of the cutoff value were considered “indeterminate” for anti-HDV IgM detection.

Qualitative detection of HDV-RNA in serum was performed as previously described by the use of a sensitive RT-PCR assay (sensitivity threshold ~100 copies/mL). HDV-RNA quantification was performed simultaneously for each patient, from serum aliquots kept frozen at −80°C, using a recently described real-time quantitative PCR assay (sensitivity threshold ~100 copies/mL). HDV genotype was determined by phylogenetic analysis of the amplified R0 region of the genome (nucleotides 885 to 1285) as previously described.

**Statistical Analysis.** The nonparametric Mann Whitney U test and Wilcoxon Rank test (StatView 4.02), and the exact Fisher t-test were used for statistical comparisons. Differences were considered significant at P values less than .05.

**Results**

**Characteristics of the Patients at Baseline (Table I).** Fourteen patients chronically infected with HBV and HDV were enrolled in this study. Patients consisted of 12 men and 2 women, aging from 28 to 52 (median age
Table 1. Baseline Characteristics of 14 Patients With Chronic Hepatitis delta

<table>
<thead>
<tr>
<th></th>
<th>n (%)</th>
<th>Median (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>12 (86)</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>42 (28-52)</td>
<td></td>
</tr>
<tr>
<td>African origin</td>
<td>6 (43)</td>
<td></td>
</tr>
<tr>
<td>French origin</td>
<td>8 (57)</td>
<td></td>
</tr>
<tr>
<td>Unknown source of infection</td>
<td>6 (43)</td>
<td></td>
</tr>
<tr>
<td>Intravenous drug use</td>
<td>7 (50)</td>
<td></td>
</tr>
<tr>
<td>Treatment with standard IFN</td>
<td>12 (86)</td>
<td></td>
</tr>
<tr>
<td>ALT level, x × normal value</td>
<td>1.95 (1.3-7.5)</td>
<td></td>
</tr>
<tr>
<td>Necroinflammatory activity</td>
<td>2 (1-3)</td>
<td></td>
</tr>
<tr>
<td>Fibrosis stage</td>
<td>3 (1-4)</td>
<td></td>
</tr>
<tr>
<td>HDV genotype 1/5b</td>
<td>12/2 (86/14)</td>
<td></td>
</tr>
<tr>
<td>Positive anti-HDV IgM</td>
<td>11 (78)</td>
<td></td>
</tr>
<tr>
<td>Positive HBV-DNA</td>
<td>4 (28)</td>
<td></td>
</tr>
<tr>
<td>HBV-DNA level, copies/mL</td>
<td>&lt;50 (&lt;50-1.9x10^6)</td>
<td></td>
</tr>
<tr>
<td>Positive HBeAg</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Positive anti-HBe</td>
<td>14 (100)</td>
<td></td>
</tr>
<tr>
<td>Positive anti-HCV</td>
<td>8 (87)</td>
<td></td>
</tr>
<tr>
<td>Follow-up duration, months</td>
<td>16 (6-42)</td>
<td></td>
</tr>
<tr>
<td>HDV-RNA level, copies/mL</td>
<td>9x10^5 (4.8x10^4 - 1.1x10^6)</td>
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</table>

aMETAVIR score.

bgenotype 1 or 5, as defined by the analysis of the R0 region of the genome.

42). Six patients were of African origin and were considered to have been infected during childhood, as no other risk factor was identified. The eight remaining patients were of French origin, and for 7 of them, past intravenous drug use was assumed to be the cause of the infection. Twelve patients (85%) had previously been treated with standard IFN (9 MU, 3 times a week) during 6 to 22 months (median treatment duration: 12 months). In all cases, this former treatment had been interrupted at least 1 year before enrollment in the present study.

All patients had abnormal ALT levels, comprised between 1.3 to 7.5 times (median 1.95) the upper limit of normal. Liver histology, assessed with META VIR (18) scoring system, indicated that most patients had moderate necroinflammatory activity (median META VIR score: 2, ranging from 1 to 3) associated to severe fibrosis (median score: 3, ranging from 1 to 4). Cirrhosis was evident in 4 patients.

HDV genotype determination indicated that 12 patients (86%) were infected by HDV-1, and 2 by HDV-5. Specific anti-HDV IgM were positive in 11 patients, negative in 1 and indeterminate in 2. HBV replication level was evaluated with a sensitive quantitative PCR assay. HBV-DNA was below the sensitivity threshold (50 copies/mL) in 10 (71%) patients, was comprised between 3.3x10^2 and 5.2x10^4 copies/mL in 3 patients, and was 1.9x10^6 copies/mL in one. In all patients, HBeAg was negative, while anti-HBe was positive.

Past and resolved HCV infection was evidenced in eight patients, who were positive for anti-HCV antibodies, but negative for the detection of HCV-RNA during at least the past 3 to 9 years.

Safety. Safety profile was similar to that usually observed with PEG-IFN alpha-2b in patients with chronic hepatitis C. Mild adverse effects such as flu-like symptoms and asthenia were observed in most patients during the first weeks of treatment but attenuated with time. Dose reduction from 1.5 to 1 μg/kg was required in five patients because of leucopenia, but no other severe side effect was noted, and no treatment withdrawal was needed.

Treatment Efficacy. The median posttreatment follow-up duration was 16 months, ranging from 6 to 42 months (Table 1). Eight patients (57%) were virological responders at the end of therapy (undetectable HDV-RNA), but 2 of them relapsed during posttreatment follow-up. Thus, 6 patients (43%) were sustained virological responders and 6 were nonresponders (43%).

Five patients (36%) were biochemical responders at the end of therapy, but 1 of them relapsed during posttreatment follow-up. However, 4 patients, who were considered biochemical nonresponders at the end of therapy achieved ALT level normalization during posttreatment follow-up. Thus, 8 patients (57%) presented a biochemical sustained response. Among them, 6 were sustained virological responders and 2 were nonresponders.

In the virological responders group, 6 patients of 8 had previously been unsuccessfully treated 12 months with standard IFN, indicating that PEG-IFN may be effective in patients with chronic hepatitis delta, even as a second line treatment. In the nonresponders group, 4 patients out of 6 required a dose reduction from 1.5 μg/kg to 1 μg/kg because of leucopenia, while in the responders group, 7 patients out of 8 received the full dose of 1.5 μg/kg.

Eight patients (3 nonresponders, 3 sustained responders and 2 responders-relapsers) underwent a second liver biopsy at the end of the treatment. META VIR scoring indicated no significant short-term histological improvement, neither for necroinflammatory activity, nor for fibrosis.

Tests to detect specific anti-HDV IgM were performed at initiation of therapy, during the course of the treatment and during follow-up. As illustrated in Table 2, the results of IgM detection did not strictly correlate those of HDV-RNA, with, for example, negative or indeterminate IgM at initiation of therapy (3 patients), or positive IgM at the end of follow-up in 2 sustained responders.

HBsAg remained positive throughout treatment and follow-up periods in all patients except one from the sustained responders group, who lost HBsAg and developed anti-HBs antibodies at month 9 of therapy. Serum HBV-
Table 2. Comparative Detection of Specific Anti-HDV IgM and HDV-RNA in Serum

<table>
<thead>
<tr>
<th>Patients (n = 14)</th>
<th>Number of patients</th>
<th>IgM / HDV-RNA detection at:</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>T0</td>
</tr>
<tr>
<td>Sustained responders (n = 6)</td>
<td>1</td>
<td>+ / +</td>
</tr>
<tr>
<td>Responder-relapser (n = 2)</td>
<td>1</td>
<td>+ / +</td>
</tr>
<tr>
<td>Nonresponders (n = 6)</td>
<td>1</td>
<td>+ / +</td>
</tr>
</tbody>
</table>

a T0: initiation of therapy, EOT: end of treatment, FU: end of follow-up
b +: Positive, -: Negative, I: indeterminate

DNA was undetectable at the end of the treatment and during the posttreatment follow-up period in all patients (responders and nonresponders) but one, who developed a symptomatic acute hepatitis (ALT level 22 folds over the normal value) 10 months after initiation of PEG-IFN. This episode occurred as HDV-RNA was becoming undetectable and was accompanied by an increase of HBV-DNA load, which exceeded $10^9$ copies/mL (Fig. 1). In this particular case, HBV-DNA load was $1.9 \times 10^6$ copies/mL at baseline and had initially been reduced by PEG-IFN therapy. PEG-IFN therapy was interrupted and was replaced by lamivudine. During the following months, HBV-DNA level decreased under lamivudine while HDV-RNA re-increased. The patient was thus considered responder-relapser to PEG-IFN for HDV infection. Taken together, these results tend to indicate that although HBV replication was low at baseline in most patients (possibly because of an inhibitory effect of HDV on HBV) the clearance of HDV-RNA under PEG-IFN therapy did not lead to the reactivation of HBV, except in one particular case, where HBV-DNA was unusually high at baseline.

Serum HCV-RNA remained undetectable in the 8 patients with anti-HCV antibodies, throughout the treatment and follow-up periods. The role of HDV on HCV replication is not well known, but previous studies have indicated a possible inhibition of HCV by HDV. The absence of HCV re-activation in the sustained responders group is probably indicative of a favorable outcome of a past HCV infection in these patients.

**Serum HDV-RNA Levels During Therapy.** At baseline, HDV-RNA levels ranged from $4.8 \times 10^4$ to $1.1 \times 10^7$ copies/mL (Table 1). Median HDV-RNA levels are shown in Fig. 2A. In the responders group (including sustained responders and relapser), HDV-RNA levels significantly decreased during the first 3 and 6 months of treatment ($P = .028$, using the nonparametric Wilcoxon Rank test). Median viral load loss was of $2.28 \log_{10}$ after 3 months of treatment, and $3.95 \log_{10}$ after 6 months (HDV-RNA being undetectable in 6 of 8 responders at M6 of treatment). In the nonresponders group, although median HDV-RNA levels decreased during therapy ($0.67 \log_{10}$ during the first 3 months, $1.84 \log_{10}$ throughout the treatment) the reduction was not significant, and viral loads re-increased after the end of the treatment. None of the nonresponders had undetectable HDV-RNA at M6 of treatment.

Median ALT levels roughly followed those of the viral loads in the responders group, but not in the group of nonresponders (Fig. 2B). Moreover, the decrease in ALT levels was never significant, even in the responders group. These results indicate that although ALT levels follow HDV viral loads during PEG-IFN therapy, they are not sufficiently accurate to monitor treatment efficiency. Individual HDV-RNA and ALT levels are represented on Fig. 3, except for 2 patients for whom the number of available frozen samples was insufficient to allow a kinetic follow-up. Figure 3 represents the four profiles of HDV-RNA and ALT levels observed during PEG-IFN therapy. Fig. 3A-B represent the typical profiles observed for the sustained responders and the nonresponders, respectively. Fig. 3C represents the profile observed for the responders-relapser. For 2 nonresponders, a distinct profile was observed (Fig. 3D): HDV-RNA significantly decreased...
during the course of the treatment (3 log_{10} copies/mL) but remained >2 log_{10} copies/mL at the end of therapy, and increased again after its ending. For all profiles, ALT levels, expressed as a multiple number of the normal upper limit value, roughly followed HDV viral kinetics. However, in some cases, moderate ALT flares were observed (Fig. 3A,D). In these examples, peaks of ALT could be explained neither by HDV nor by HBV replication rebounds.

**Predictive Factors of Response.** The limited number of patients included in this study did not allow us to determine accurate predictive factors of response to PEG-IFN treatment. In particular, epidemiological data such as the geographic origin (France or Africa), the HDV genotype (HDV-1 or HDV-5), the mode of contamination (unknown or use of intravenous drugs), or the age at the initiation of treatment could not be taken into account for statistical analysis. However, our results indicate that HDV-RNA kinetics might provide predictive information concerning the response to PEG-IFN. Indeed, after 3 months of PEG-IFN, HDV-RNA levels were significantly lower in the responders than in the group of nonresponders ($P = .018$ by the use of the nonparametric Mann-Whitney U test). This trend was confirmed after 6 months of PEG-IFN ($P = .006$, with 75% of the responders being undetectable for HDV-RNA, versus none in the nonresponders group. Moreover, negative HDV-RNA at M6 was predictive of sustained response ($P = .021$). At baseline, neither HDV-RNA levels, nor ALT levels, IgM detection or histological grade were predictive of treatment response.

**Discussion**

In this study, 14 patients with chronic hepatitis delta received 12 months of PEG-IFN alpha-2b, and a sustained virological response was achieved in 6 patients (43%). In 2 recent studies evaluating the virological response to standard IFN therapy by the means of qualitative RT-PCR assays with sensitivity thresholds similar to the one we describe, a sustained virological response was obtained after two years of IFN monotherapy (9 MU three times weekly) in 20% of the patients. Thus, our results seem to indicate that PEG-IFN is probably at least as effective as standard IFN for treatment of chronic hepatitis delta, with sustained responses obtained after one year of treatment. Moreover, we observed that the tolerance profile was not different from that described during the treatment of chronic hepatitis C although 5 (36%) patients required a dose reduction because of leucopenia. Interestingly, 4 of these patients were nonresponders, suggesting that virological response might be related to the dose ($P = .036$). As previously suggested by a single case of an HIV positive patient with chronic hepatitis delta, we believe that the use of PEG-IFN could be extended to the treatment of hepatitis delta, and that its efficacy should be compared to that of standard IFN by the means of large scale comparative studies.

We monitored the efficacy of PEG-IFN by the means of biochemical, serological and molecular analyses. HDV-RNA was detected by the means of a very sensitive qualitative RT-PCR, quantification being secondarily performed from frozen aliquots of the sera. We found that the most reliable biological marker for treatment monitoring was HDV-RNA quantification in serum. Indeed, we detected flares of cytolysis that were independent of HDV replication (as illustrated in Fig. 3A,C-D), but might have been generated by the treatment itself, as previously described in chronic hepatitis B. Moreover, like others, we observed that anti-HDV IgM could re-
main undetected despite HDV replication, or, on the contrary, could be detected while no HDV-RNA was detected in the serum (Table 2). Therefore, as previously suggested, and despite the usefulness of tests to detect anti-HDV IgM, we recommend the use of a consensus and sensitive RT-PCR approach for treatment monitoring of chronic hepatitis delta.

Besides qualitative detection, quantification of HDV-RNA provides useful information regarding the individual viral load kinetics during treatment, and might be taken into account for the adjustment of treatment duration. Indeed in most studies, interferon is administered during 12 months although some authors adopt longer regimens or adapt treatment length to individual factors. In our study, most patients of the responder group had achieved HDV-RNA clearance during the first 6 months of treatment. However, the fact that 2 of them relapsed after the end of the treatment seems to indicate that PEG-IFN might need to be continued for more than 12 months, if possible. Four patients of the nonresponders group kept a viral load decrease <2 log_{10} copies/mL throughout the 12 months therapy. In such cases of insignificant viral load decrease after a period of time which remains to be defined (probably ranging between 9 and 12 months of treatment), we suggest that a change in the treatment schedule might be considered, depending on the tolerance and on the effect of the treatment upon liver fibrosis. In 2 patients of the non responders group (Fig. 3D), the kinetic profiles looked more like those of the group of responder-relapsers (Fig. 3C) than like those of the nonresponders (Fig. 3B), suggesting the existence of slow responders among patients considered nonresponders after only 12 months of treatment. When slow responders can be identified on the basis of HDV-RNA kinetics, long-term treatment may need to be considered. Further studies implying a greater number of patients are...
needed to specify these preliminary suggestions. Considering the small number of patients followed in most specialized care centers, large-scale multicenter studies are required to define guidelines for optimal management of patients with chronic hepatitis delta.

In this study, the course of HBV infection was also considered because of the specific relationship between HBV and HDV during chronic hepatitis delta. Indeed, although HDV depends on HBV to produce infectious viral particles, it also inhibits HBV replication in vitro as well as in vivo. Moreover, PEG-IFN, which was used to treat hepatitis delta is also efficient to treat HBV infection, antiviral therapy combining PEG-IFN with an HBV replication inhibitor may need to be considered when HDV infection is associated to active HBV replication.

In conclusion, this preliminary study indicates that PEG-IFN is well tolerated and effective in the treatment of chronic hepatitis delta, even in the case of a previous failure of standard IFN therapy. Controlled studies are needed to evaluate the benefit of PEG-IFN against standard IFN, and to define the optimal treatment schedule. The quantitative detection of HDV-RNA in serum improves treatment monitoring, and the analysis of the HDV-RNA kinetic profiles might help to adjust the duration of therapy. Studies of larger scale are needed to specify the predictive value of early virological response.

References


