Clinical Studies

Treatment of chronic hepatitis delta with pegylated interferon-α2b

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Abstract: Background/Aims: Chronic hepatitis D is difficult to treat. The present pilot study investigated the efficacy and tolerability of pegylated (PEG)-interferon (IFN)-α2b in chronic hepatitis D. Patients and Methods: Twelve patients with chronic hepatitis D were prospectively treated with 1.5 μg/kg PEG-IFN-α2b for 48 weeks and followed for 24 weeks. Sustained response (SR) was defined as undetectable hepatitis delta virus (HDV) RNA by reverse transcriptase-polymerase chain reaction and normalization of alanine aminotransferase (ALT) at 6 months after treatment. Investigations included HDV RNA kinetics, determination of hepatitis B virus (HBV) and HDV genotypes and histological evaluation. Results: An SR was achieved in two out of 12 of patients (17%). The negative predictive value of a less than 3 log HDV RNA decrease at month 6 was 100%. The positive predictive value of a more than 3 log HDV RNA decrease at month 6 was 67%. A marked ALT reduction at the end of treatment was observed in responders and nonresponders. Ishak histological score was comparable at baseline and significantly improved in responders compared with nonresponders at the end of follow-up (13.5 vs. 8.0; P<0.02). Conclusion: The present study indicates that PEG-IFN-α2b is a promising treatment option in chronic hepatitis D. Nonresponders could be identified by a less than 3 log decrease of HDV RNA at 6 months of treatment.

Hepatitis delta virus (HDV) infection affects about 15 million persons worldwide (1). Although hepatitis delta is a rare disease compared with hepatitis B or hepatitis C, its importance results from a high morbidity and mortality. Acute hepatitis delta can lead to severe liver disease, with the highest rate of fulminating courses among the hepatotropic viruses (2). Superinfection of hepatitis B virus carriers with HDV leads to chronic hepatitis in about 90% of cases. Compared with chronic hepatitis C and B, progression to liver cirrhosis has been reported to be more frequent and rapid in chronic hepatitis delta (3). Furthermore, the incidence of hepatocellular carcinoma is increased in patients with hepatitis delta compared with patients with hepatitis B virus (HBV) monoinfection (3).

So far, there is no effective treatment of chronic hepatitis D. Sustained virus elimination can be achieved by high-dose or prolonged (12–24 months) interferon (IFN) therapy in less than 10% of HDV-infected patients when sensitive reverse transcriptase-polymerase chain reaction (RT-PCR) testing is used as an endpoint (4, 5). However, because of preexisting liver cirrhosis and significant side effects, many patients do not tolerate high IFN doses. A biochemical response and histological improvement has been reported in 7–70% of patients at the end of an antiviral treatment and in 0–43% at the end of follow-up (6–10). Long-term IFN treatment beyond 12 months might be beneficial in chronic hepatitis D (11, 12). In a case report, treatment with standard IFN for 12 years was able to induce a sustained virus suppression in chronic HDV superinfection (13). Persistent alanine aminotransferase (ALT) normalization can be associated with a regression of liver fibrosis despite ongoing viral replication (9, 11, 14).
Lamivudine or ribavirin monotherapies and lamivudine–IFN combination therapies have not been proven to be effective in chronic hepatitis D (15–18). In view of the inefficiency of lamivudine, it is unlikely that adefovir, tenofovir or other nucleos(t)ide analogues might be useful for the treatment of hepatitis D. Prenylation inhibitors are promising HDV-specific agents but have not been studied in humans so far (19).

Long-term follow-up in patients with hepatitis delta has been performed in a limited number of smaller studies (3, 20–23). A beneficial effect of an antiviral therapy on the long-term clinical course of hepatitis D has been reported for patients who received a high-dose IFN treatment independent of the sustained virological response at 6 months after the end of treatment (14).

PEG-IFNs have been reported to be superior to standard IFNs with regard to sustained virological response in chronic hepatitis B and C (24, 25). The aim of the present pilot study was to investigate the efficacy and safety of PEG-IFN-α2b in chronic hepatitis D.

Patients and methods

A total of 12 patients with chronic hepatitis D have been included in a prospective study. Patients were treated with PEG-IFN-α2b s.c. at a high dose of 1.5 μg/kg for 48 weeks given the high doses of standard IFN recommended in chronic hepatitis D. Patients were followed for another 24 weeks after termination of IFN treatment. Combined ALT normalization and negativation of HDV RNA at 24 weeks of follow-up was defined as sustained response (SR). Informed consent was obtained from each patient. The study was approved by the local ethics committee and performed according to the ethical guidelines of the 1975 Declaration of Helsinki.

The clinical, biochemical and histological characteristics of patients are given in Tables 1 and 2. Previous IFN therapy had been performed in three patients, but IFN dose or treatment duration had been insufficient. Treatment had been performed with 3 MU IFN-α thrice weekly in two patients and for less than 4 months in one patient. The diagnosis of chronic hepatitis D was based on commonly accepted criteria. All patients had HBsAg (Abbott Laboratories, Wiesbaden, Germany) and detectable HDV antibodies for more than 6 months (Ortho Diagnostics, Tokyo, Japan). HDV infection was confirmed by qualitative HDV RNA RT-PCR in all patients at baseline. All 12 patients with chronic hepatitis delta were HBV DNA negative in a commercial HBV DNA hybridization assay (Versant HBV DNA 3.0, Bayer, Leverkusen, Germany). However, seven patients tested positive for HBV DNA by PCR.

All patients were tested negative for HIV by ELISA. Two patients tested positive for anti-HCV by ELISA but were negative for HCV RNA (bDNA Assay 3.0, Bayer). Other liver diseases had been excluded.

HBV genotyping

HBV genotype was determined in seven patients by direct sequencing using primers spanning part of the HBV surface gene (primer sense 5’-tggatgtgtgctggc-3’; primer antisense 5’-cKttgaca-Dactttcaataatg-3’). HBV DNA isolation from serum was performed with a commercial kit (High Pure 16 System Viral Nucleic Acid kit; Roche Diagnostics, Mannheim, Germany). PCR amplification on a LightCycler (Roche Diagnostics, Mannheim, Germany) was performed with 50 cycles after an initial denaturing step at 95°C, an annealing temperature of 69–60°C (‘touch down PCR’) and an elongation temperature of 72°C. Sequencing was performed as described previously (26). HBV DNA could not be amplified by PCR in five patients because of the absence of viremia.

HDV RNA quantification and HDV genotyping

HDV RNA was isolated from 400 μl serum with an automatic nucleic acid extractor and a com-
commercial isolation kit (MagNA Pure Compact Nucleic Acid Isolation Kit I, Roche Diagnostics). Reverse transcription and PCR were performed on a LightCycler with each 10 pmol of the HDV sense primer (5'-tccagagaccctca-3') and HDV antisense primer (5'-ccgggataagcact-3') using a commercial kit (LightCycler RNA amplification kit for hybridization probes; Roche). Reverse transcription was carried out at 55°C for 20 min. DNA amplification was performed as touchdown PCR with an initial annealing temperature of 62 °C tapering down to 48 °C using a fluorescein hybridization probe for HDV genotype I/II (5'-gagacagaggggaggaag-FL-3') and a 5'-LC 640 nm aaagaRagcaRccggctagc-3' hybridization probe. A patient's serum that had been calibrated on the HDV plasmid pSVL LD3 was taken as the standard (27). The diagnostic sensitivity of the HDV RNA assay was 150 copies/ml. For HDV genotype III, the 5'-gaagccgagactgggaagag-FL-3' probe was used. Quantitative HDV RT-PCR could not be performed in one patient.

Liver histology

Baseline biopsies were available in 11 patients. One patient did not agree to liver biopsy. Scoring of liver histology was performed according to the classification of Ishak et al. (28) by a blinded pathologist (A. D.). A second liver biopsy 6 months after the end of treatment was obtained in seven patients (two responders and five non-responders).

Statistics

Student's t-test and \( \chi^2 \) test were used to analyze the data. Statistical analysis was performed by using the SPSS program (SPSS Inc., Munich, Germany).

### Results

**Baseline characteristics**

Male gender and eastern European origin predominated among patients with hepatitis delta. Blood transfusions, a positive family history and former intravenous drug abuse were the most common risk factors for acquisition of hepatitis delta (Table 1). Diagnosis of hepatitis B preceded that of hepatitis delta by about 7 years. Despite the young age of the patients (34 ± 15 years), incomplete cirrhosis was histologically proven in three out of 11 patients (27%) at initial presentation. Hepatitis D genotype I or II was prevalent among the patients; there was no carrier of HDV genotype III. All patients with amplifiable HBV DNA (\( N = 7 \)) carried the HBV genotype D.

**PEG-IFN treatment**

Patients received a mean cumulative dose of 4169 ± 1963 µg of PEG-IFN-α2b. The dose of the PEG-IFN-α2b had to be reduced in four of 12 patients because of thrombocytopenia. Overall there was a decrease in ALT levels during PEG-IFN therapy with a significant reduction in ALT levels at the end of treatment (Table 2), but mean ALT levels increased again after treatment cessation. ALT decrease was most pronounced in sustained responders, but also nonresponders displayed a decrease in ALT levels from baseline to the end of treatment (68 ± 33 vs. 40 ± 32 U/l) although significance was not reached (Fig. 1). A 50% reduction in ALT levels at the end of treatment compared with baseline was observed among four out of 10 nonresponders. There was no correlation between the ALT decrease and HDV RNA decline in the nonresponders. At the end of follow-up, still two out of 10 nonresponders displayed a 50% decrease in ALT. A normalization of ALT at the end of treatment was achieved in 27% and a negativation of HDV RNA in 17% of patients. Normalization of ALT and negativation of HDV RNA was sustained in 17% of the patients (sustained responders). A more than 3 log decrease in HDV RNA was identified in responders at month 6 (Fig. 2A), but only in one out of nine nonresponders (Fig. 2B and C). The negative predictive value of a less than 3 log HDV RNA decrease at month 6 was 100%; the positive predictive value of a 3 log or more decrease of HDV RNA at month 6 was 67%. The two responders displayed lower baseline HDV RNA levels (7.8 \( \times \) 10^6 vs. 3.0 \( \times \) 10^5 copies/ml) and slightly higher ALT levels (91 ± 58 vs. 68 ± 33 U/l) than nonresponder patients.
Ishak score at baseline was not different between responders and nonresponders. A second liver biopsy was available in seven out of 11 patients (64%). An improvement in the total Ishak score in the second liver biopsy was seen for the two responders compared with the 10 nonresponders. However, no relevant deterioration in the Ishak total score and subscores from the baseline level was noticed in the nonresponder patients (Fig. 3). All patients with liver cirrhosis (n = 3) were nonresponders.

No serious adverse events were noted in the patients. Flu-like symptoms and local skin reaction at the injection site were the most frequently reported side effects (Table 3). Marked thrombocytopenia was the most common reason for dose reduction, which can be explained by the high rate of patients with advanced fibrosis and cirrhosis.

Discussion

Chronic hepatitis D is difficult to treat. Treatment regimens for hepatitis D are IFN based so far (4, 5). The response to standard IFN is better with high doses of IFN (9–10 MU thrice weekly) than lower IFN doses (9). Furthermore, prolongation of treatment beyond 12 months appears to be beneficial (11–13).

Sustained virological response to IFN varies between 0% and 43% in the studies reported so far (5). However, no sustained virological response was achieved in the only randomized study that measured HDV RNA by a sensitive RT-PCR method (14). One nonrandomized study using RT-PCR measurement reported a sustained virological response of 10% among 21 patients treated for 24 months (12). In the present study, a sustained virological response was achieved in 17% of patients, which is superior to the historical response rates of former studies using RT-PCR determination of HDV RNA.

No predictive factors for response to IFN have been identified so far in chronic hepatitis D. Virus kinetics performed in the present study suggest that patients with a less than 3 log decrease at month 6 are unlikely to become sustained virological or biochemical responders. Virus load determination at month 6 might be helpful for establishing a stopping rule for treatment discontinuation.

Baseline parameters were not as suited as virus kinetics for prediction of IFN response. There is increasing evidence that HBV genotypes play a
Table 3. Side effects and adverse events of pegylated interferon (PEG-IFN)-α2b

<table>
<thead>
<tr>
<th>Side effect</th>
<th>PEG-IFN (N=12)</th>
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</thead>
<tbody>
<tr>
<td>Flu-like symptoms</td>
<td>7</td>
</tr>
<tr>
<td>Local skin reaction (&gt;3 cm)</td>
<td>5</td>
</tr>
<tr>
<td>Weight loss (5–10%)</td>
<td>5</td>
</tr>
<tr>
<td>Fatigue</td>
<td>4</td>
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<tr>
<td>Diffuse loss of hair</td>
<td>4</td>
</tr>
<tr>
<td>Abdominal pain</td>
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<td>Thrombocytopenia</td>
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