Efficacy and Safety of Peginterferon alfa-2a (40KD) in HBeAg-positive Chronic Hepatitis B Patients

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Abstract

Aim: The study was designed to evaluate the efficacy and safety of peginterferon α-2a in HBeAg-positive chronic hepatitis B patients, nonresponders or relapsers after previous lamivudine or standard interferon therapy. Methods: This prospective, national, multicentric, open label, not randomized trial enrolled 43 HBeAg-positive chronic hepatitis B patients with detectable HBsAg for at least 6 months prior to screening, positive HBeAg and negative anti-HBe, serum HBV DNA levels of at least 500,000 copies/mL by PCR assay, elevated ALT up to 10 x ULN, no response or relapse after previous lamivudine or standard interferon therapy. All eligible patients received pegIFN α-2a 180 μg weekly for 48 weeks with 24 weeks treatment free follow-up. There were two main efficacy assessments: HBeAg seroconversion and viral suppression below 100,000 copies/mL. Results: HBeAg seroconversion rate at the end-of-treatment was 4.65% (n=2; p<0.05) increasing to 11.62% 24 weeks after end of therapy (n=5; p<0.05). The rate of viral suppression at levels below 100,000 copies/mL was 23.25% (n=10; p<0.05) at end-of-treatment, and 16.3% (n=7; p<0.05) at end of follow-up. ALT normalization was obtained in 20.9% (p<0.05) of patients at end-of-treatment, the percentage being significantly higher - 37.2% (p<0.05) at the end of follow-up. Conclusions: Even in a difficult-to-treat patient population with HBeAg-positive chronic hepatitis B, peginterferon alfa 2a proved to be efficient in a defined proportion of patients. The increase in HBeAg seroconversion rate from end-of-treatment (4.65%) to the end of follow-up period (11.62%) also proves the benefits of prolonged immunological effect of pegIFN α-2a.

Key words

Alanine aminotransferase – chronic hepatitis B – hepatitis B e antigen – hepatitis B virus – interferon α-2a – viral DNA.

Introduction

Hepatitis B virus (HBV) is one of the most frequent viruses in humans; almost a third of the world population has serological biomarkers of infection and approximately 350 million patients suffer from chronic infection with HBV [1, 2]. Chronic hepatitis B is an inflammatory liver disease of variable severity associated with a significantly increased risk of cirrhosis, liver failure and hepatocellular carcinoma. Although HBV is a non-cytopathic virus, the liver injury is mainly mediated by the host immune response against infected hepatocytes and by the production of inflammatory cytokines [2, 3].

The suppression of HBV replication before established significant irreversible liver damage is the therapeutic goal of chronic viral hepatitis B treatment. Ideally, therapy aims to suppress viral replication and to induce remission of liver disease [4, 5]. The long-term goals of treatment are virological clearance, delayed progression to cirrhosis or liver cancer and increased survival [6].

The response to antiviral therapy could be classified in three categories: biochemical, virological and histological. In initially HBeAg positive-patients effective treatment of chronic HBV is defined in terms of sustained clearance of circulating HBeAg with development of HBe antibodies and HBV-DNA decline to levels below 100,000 copies/mL. The improvement of liver disease could be assessed by documenting the normalization of serum alanine transaminase levels (biochemical response), at least a two point reduction of necroinflammatory index and stable fibrosis score (no worsening) on liver biopsies (histological response). Furthermore, loss of HBsAg is associated with improved survival and reduced risk of HCC. Although loss of HBsAg is clearly the ultimate goal aimed at achieving by
therapy in a HBV-related disease and is therefore a useful surrogate endpoint, its relatively rare occurrence limits its utility for evaluation of new therapies [4].

Ultimately effective treatment is necessary to prevent the progression of chronic hepatitis B to cirrhosis, liver failure, hepatocellular carcinoma, and death and subsequently improve a patient’s quality of life and survival [7].

Currently, seven antiviral agents are approved for the treatment of chronic hepatitis B: conventional interferon (IFN) alfa, pegylated IFN (PEG-IFN) alfa, lamivudine, adefovir dipivoxil, entecavir, telbivudine and tenofovir [8, 9]. Conventional IFN alfa has suboptimal pharmacokinetics, resulting in a frequent dosing schedule and variable drug exposure. Lamivudine, telbivudine and to a lesser extent adefovir are also associated with drug resistance [10, 11], mainly in long-term use. PEG-IFN alfa was developed through a pegylation process where a polyethylene glycol (PEG) polymer molecule is attached to the conventional IFN-α molecule to produce a molecule with improved pharmacokinetics, namely a prolonged half-life.

Since PEG-IFN alfa treatment induces an improved virologic response in chronic hepatitis C patients, the question naturally arises whether PEG-IFNs would be useful in patients with chronic hepatitis B. Evidence that higher doses of IFN are more effective supports this approach. The established disadvantages of lamivudine, the potential development of resistance mutations in hepatitis B virus patients treated with different antiviral agents (particularly as single agents), as well as the acknowledgement of the fact that HBe seroconversion is a critical step to establish the duration of treatment, all of these could offer a major role of PEG-IFNs in the treatment of chronic HBV infection [7, 8, 12, 13].

In 2005, PEG-IFN α-2a (Pegasys®) was approved for use in patients with chronic hepatitis B in the US, the EU, Switzerland, Turkey and in several countries in the Asia-Pacific region. Several trials were carried out to compare PEG-IFN α-2a with conventional IFN or lamivudine in monotherapy or with PEG-IFN plus lamivudine combination in patients with HBeAg-positive and negative chronic hepatitis B [14-16]. PEG-IFN α-2a proved to be more efficient than conventional IFN in both HBeAg-positive and negative patients. Although there is an increased viral suppression throughout the treatment period, the combination of lamivudine and PEG-IFN α-2a does not improve sustained viral response rates at the completion of the 24-week follow-up after the treatment period, as compared to PEG-IFN α-2a monotherapy [17]. PEG-IFN α-2a adverse events profile was reasonable. Thus, PEG-IFN α-2a is the first line treatment in many patients with either HBeAg-positive or HBeAg-negative chronic hepatitis B; there is also room for therapeutic failures to be retreated with other antiviral agents such as nucleotide and nucleoside analogues.

This study was designed to assess the efficacy and safety of PEGASYS® 48-week monotherapy in HBeAg positive patients with chronic hepatitis B who failed to respond or relapsed on previous treatment with lamivudine or conventional IFN.

**Methods**

**Study design**

This was a multi-centre, national, open-label, non-randomized study; all enrolled patients received 48-week PEGASYS® as a single therapy and then were followed-up over 24 weeks. The study was conducted in 8 Romanian health centers between 2005 and 2007 and was designed by the sponsor (Roche) in collaboration with hepatology specialists. All patients were screened within 35 days prior to study drug initiation. This was a 72-week study comprising 48 weeks of treatment and 24 weeks of follow-up. A dose of 180 mcg of PEGASYS® was administered s.c. every week, for 48 weeks. At each visit, the dose could be adjusted as required by the occurrence of certain adverse events. The study was conducted in compliance with the Helsinki Declaration and with the Good Clinical Practice principles. All patients had to sign an informed consent form, before any procedure of the study was performed.

**Patients**

In total, 43 patients were enrolled. Subjects were considered eligible if they fulfilled all the following criteria: age between 15 and 65 years; HBsAg positive for at least 6 months; HBsAb negative and HBeAg positive for at least 6 months; HBV DNA > 500,000 copies / mL by PCR, a serum alanine aminotransferase (ALT) level within the range of >1 x ULN and ≤10 x ULN, prior liver biopsy findings consistent with chronic hepatitis B (optional), and no response/relapse after previous treatment with LM or IFN.

Exclusion criteria included evidence of decompensated liver disease (Child-Pugh score ≥5), co-infection with HAV, HCV, HDV and/or HIV, comorbidities or severe psychiatric diseases, evidence of severe retinopathy, a neutrophil absolute count < 1500/mm^3, a platelet count <90,000/mm^3, hemoglobin < 11.5 g/dL for females and 12.5 g/dL for men, a serum creatinine level > 1.5 x ULN, serum bilirubin > 2 x ULN, evidence of drug abuse (including excessive alcohol consumption) within one year prior to study entry and inability or unwillingness to provide an informed consent or to comply with the study requirements.

Patients must not have received any antiviral treatment (IFN or analogues) for their chronic hepatitis B within 6 months before study entry. Systemic antiviral, anti-neoplastic and immunomodulatory treatments (including steroids and radiotherapy) were not allowed over the entire study period.

**Main efficacy measures**

Treatment efficacy analysis includes all screened patients receiving at least one dose of the study medication. The study had two prespecified primary measures of efficacy assessed after 24 weeks of treatment-free follow-up: HBe seroconversion rates (defined in terms of HBeAg loss and HBeAb production) and suppression of viral replication
Peginterferon alfa-2a in HBeAg-positive chronic hepatitis B patients

Secondary efficacy measures at the end of treatment (EOT) and at the end of the 24 weeks follow-up (end of follow-up, EFU) included HBeAg loss, HBV-DNA suppression below the detection limit as measured by Roche PCR tests, normalization of ALT levels, HBsAg loss with the appearance of HBsAb and mixed response (HBeAg loss, undetectable levels of HBV DNA by Roche PCR tests and transaminase level normalization).

Safety analysis

Safety assessment measures included adverse events, hematologic and chemical measurements, and vital signs. The severity of adverse events was graded on a four-point scale (mild, moderate, severe and life-threatening), while causality was determined by the investigator. The safety analysis included all randomized patients who had received at least one dose of study medication and who had undergone at least one safety assessment after baseline.

Statistical analysis

The demographics were distributed according to mean, medium, standard deviation, maximum and minimum value for a certain variable and in frequency and percentage tables for different variable categories.

The response rates for primary and secondary endpoints were displayed in percentages with a 95% confidence interval.

The \( t \) test was used to test the percentage difference, while the chi-square test was used to compare the ensuing percentages. The means were compared using the Independent Sample T Test, the Paired Sample T Test and the Nonparametric Tests (2 Independent Samples and 2 Related Samples).

Results

Patients characteristics

Forty-three patients were enrolled, 27 (62.8%) males and 16 (37.2%) females with a mean age of 31 years (see Table I). Of these, 72.1% had been previously treated with conventional IFN and 93% with Lamivudine. One single diagnosis of cirrhosis was documented at screening.

The patients had been off therapy before trial enrollment for a period between 6 and 109 months. Response to previous therapy (non-responder or relapser) before study as well as lamivudine resistance in lamivudine–pretreated patients were not assessed.

Virologic response

At the completion of the treatment period, the proportion of patients with HBV DNA <100,000 copies/mL amounted to 23.25% (n=10). This percentage decreased during the follow-up period, reaching 16.3% at its completion (Table II). In patients with HBV DNA levels below 100,000 copies /mL at EFU, there was a considerable 3 log decrease of HBV DNA from baseline at EOT.

This trend was maintained throughout the follow-up period; we recorded an almost 4 log decrease from baseline at the end of this period (Fig. 1).

Comparing the mean HBV DNA (log copies/ml) in all study patients we noticed a significant 1.50 log EOT decline from baseline (8 vs. 6.35, \( p=0.00 \)). During follow-up, there was a 0.5 log statistically significant increase in the mean HBV DNA (6.35 vs. 6.89, \( p=0.03 \)) (Fig. 2).

In the group of patients with HBV DNA >100,000 copies/mL at the EOT, the EOT mean value of the viral load was significantly decreased from baseline (8.19 vs 7.36 log, \( p=0.05 \)) (Fig. 3).

Comparing the HBV DNA drop in patients with or without virological, response, we found that if at baseline, the mean values of HBV DNA (log) were similar (7.8 vs. 7.93, \( p=0.428 \), on-treatment suppression patterns were very

### Table I. Baseline characteristics of patients with chronic hepatitis B (N=43)

<table>
<thead>
<tr>
<th>Table I. Baseline characteristics of patients with chronic hepatitis B (N=43)</th>
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<tbody>
<tr>
<td>Sex</td>
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<td>Male - no (%)</td>
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<td>Female -no (%)</td>
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<td>Age - yr</td>
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<td>Mean +/-SD</td>
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<td>Minimum</td>
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<td>Maximum</td>
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<td>Diagnosis of chronic hepatitis B - months</td>
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<td>Mean</td>
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<td>Median</td>
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<tr>
<td>Minimum</td>
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<td>Maximum</td>
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<tr>
<td>Previous use of antiviral treatment IFN - no (%)</td>
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<td>Duration of IFN - months</td>
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<tr>
<td>Previous use of antiviral treatment lamivudine - no (%)</td>
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<tr>
<td>Duration of lamivudine - months</td>
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<tr>
<td>HBV DNA - log</td>
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<td>Minimum</td>
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<tr>
<td>Cirrhosis - no (%)</td>
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<td>AFP level</td>
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<td>Minimum</td>
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<td>Maximum</td>
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<td>ALT (U/L)</td>
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HBV DNA (log) levels over the 72 weeks study period patients with HBV DNA < 100,000 copies/mL at the end of follow-up. At the end of the follow-up the mean values of HBV DNA (log) were significantly different (3.99 vs. 7.64, p=0.00) (Fig. 4).

**HBeAg response**

The percentage of patients with HBe seroconversion at the end of the treatment (week 48) was 4.65%. The overall Hbe seroconversion rate continued to rise throughout the study and was higher at EFU: 11.62% (Table II).

**Alanine aminotransferase levels**

At the end of treatment (week 48), the proportion of patients with normalized ALT levels was 20.9%, CI: [8.75-}

### Table II. Primary efficacy of the therapy

<table>
<thead>
<tr>
<th>Period</th>
<th>End of Treatment (over weeks 12–24 of follow-up)</th>
<th>Stable response</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV DNA &lt;100,000 copies/mL</td>
<td>10 (23.25%) 7 (16.3%)</td>
<td>3 (6.97%) maintained HBV DNA below 100,000 copies/mL both EOT and EFU</td>
</tr>
<tr>
<td>HBeAg seroconversion</td>
<td>2 (4.65%) 5 (11.62%)</td>
<td>Both patients with HBeAg seroconversion at the EOT maintained this response at the EUP</td>
</tr>
</tbody>
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Fig. 1. HBV DNA (log) levels over the 72 weeks study period patients with HBV DNA < 100,000 copies/mL at the end of follow-up.

Fig. 2. Mean HBV DNA (log copies/ml).

Fig. 3. Mean HBV DNA (log copies/mL). Patients with HBV DNA > 100,000 copies/mL.

Fig. 4. HBV DNA (log) levels over the 72 weeks study period. Patients with HBV DNA > 100,000 copies/mL vs patients with HBV DNA < 100,000 copies/mL.
33.05], p<0.05. During follow-up, the percentage of patients with normalized ALT levels was significantly higher: 37.2%, CI [22.75-51.65], p<0.05.

Mean ALT in patients with EFU normalized ALT decreased significantly after week 2 of treatment; the EOT mean ALT was 76.5 U/L as compared to 135.1 U/L (43.3% decrease). This reduction was maintained between EOT and EFU (76.5 U/L vs. 58.3 U/L, p=0.057) (Fig. 5).

Mean ALT (U/L) decreased significantly between baseline and end of treatment (p=0.03). The decrease is more marked if we compare baseline and EFU mean ALT (p=0.02) (Fig. 6).

There is a strong correlation between the decrease of HBV DNA and ALT normalization, all the patients with HBV DNA < 100,000 copies/mL registering normalized ALT level (p=0.00).

Safety

The overall withdrawal rate was low (16.3%), 9.3% due to safety reasons and 7% due to other reasons. PEGASYS® dose reduction occurred in 20.9% of patients and the majority of these adjustments were caused by neutropenia and thrombocytopenia (Table III).

There were 10 patients (23.2%) who had a dose reduction of peginterferon alfa-2a due to safety reasons. The majority of these patients had a dose reduction of 135 mcg (6.95%).

The rate of non-serious adverse events was 44.2% while the rate of serious adverse events was significantly lower (11.6%) (Table IV).

Depression, potentially induced by IFN-based therapy, was infrequent during the study, being reported by 1 patient (2.32%) treated with PEGASYS.

Discussion

Due to the limitations of actual treatment options, it is generally accepted that eligible patients for antiviral treatment with IFN (conventional or pegylated) and / or nucleoside / nucleotide analogues (NAs) should be in immune clearance or in reactivation phase of chronic HBV infection, those patients having the best chance for response.

The primary objective of antiviral treatment is stopping...
or delaying disease progression to cirrhosis, decompensated cirrhosis or hepatocellular carcinoma, with prolongation of survival and life quality improvement. To achieve this goal, secondary objectives must be achieved first (serologic, virologic, histological and biochemical), which differ in importance and priority for HBe positive and HBe negative chronic hepatitis patients, respectively.

There are a number of predictive factors for response to treatment, no matter the treatment choice (PEG-IFN or NAs), these factors should be evaluated at baseline and during treatment.

Predictive factors for serologic (HBe and HBs seroconversion) and virologic (undetectable HBV-DNA) response to IFN are: HBV–DNA below 10^5 IU/mL, high level of ALT (over 3 x ULN), high necroinflammatory activity at baseline and genotype A, all of them associated with best therapeutic results [7].

Regarding patients enrolled in this study, it must be mentioned this was a difficult-to-treat study population, since all patients had failed or relapsed after a previous course of therapy with lamivudine or conventional IFN. Concomitantly, recent Romanian epidemiological data on HBV genotype prevalence show that genotype D (with the lowest response rates to IFN based therapy) could be identified in almost 80% of patients with chronic HBV infection while genotype A accounts for less than 13% of the cases [20].

Considering all the above, the results of our study prove that PEG-IFN alfa-2a is efficient and safe for the treatment of HBeAg-positive chronic hepatitis B in a defined proportion of patients with relapse or non-response to previous lamivudine or conventional IFN therapy. 16.3% of the study patients managed to achieve HBV DNA levels < 100,000 copies/mL. Overall, there was a 4-fold (copies/mL) decrease from baseline in the mean value of HBV DNA (copies/mL) at the EFU. Suppression of viral replication at lowest possible levels is crucial for future clinical disease evolution, ensuring achievement of therapeutic objectives but also lowering the risk for hepatocellular carcinoma as proved by disease natural history studies (REVEAL study).

HBe seroconversion is the key objective of HBeAg-positive chronic hepatitis B therapy, as it was associated with better long-term outcomes, such as response sustainability, histologic improvement and increased complication-free and overall survival [18]. HBe seroconversion is the best goal for HBe positive chronic hepatitis B patients because almost two thirds of patients achieving seroconversion will go into the “inactive carrier” phase, with minimal active disease and relatively stable evolution after therapy cessation. For the rest of patients, viral mutations will develop in “precore” or “core promoter” domain, with a decrease or lack of HBeAg expression and histological active chronic hepatitis. HBe seroconversion is a dynamic process, with a higher risk for seroreversion after NA treatment (10 to 25% patients in 3 years after treatment). Interferon treatment demonstrated the best rate of HBe seroconversion (32% after a fixed period of treatment). Both drug induced seroconversion (after IFN or NA) are less durable compared to spontaneous seroconversion.

Although the 4.65% percentage of patients with EOT HBe seroconversion may seem rather low compared to the results reported so far in HBeAg positive (but naïve) patients, it is interesting to note that it did increase significantly during the treatment free follow-up weeks and amounted to 11.62% at the end of this period. There was a stable treatment response in all patients with EOT HBe seroconversion. Still, due to seroreversion risk, HBe seroconversion should not be considered the final treatment goal. Monitoring is mandatory in the following time after treatment.

According to the analysis of patients with HBV DNA <100,000 copies/mL, they had a significantly higher baseline level of ALT than patients with an EFU viral load >100,000 copies/mL. On the other hand, most patients with HBV DNA <100,000 copies/mL during follow-up had ALT levels greater than 2 x ULN at treatment initiation. Patients whose viral load did not reach levels <100,000 copies/mL had baseline ALT levels less than 2xULN; this observation supports the concept that baseline ALT level is one of the predictive factors of patients’ response to IFN treatment [18, 19]. In contrast, no statistically significant differences between groups (HBV ADN <100,000 copies/mL or >100,000 copies/mL) were observed for the duration of hepatitis, age, sex, type and duration of previous antiviral treatment (lamivudine or IFN).

Patients with a biochemical response had a significantly lower mean HBV DNA (log) compared to patients without ALT normalization at the EFU. Baseline ALT levels did not differ between patients with and without biochemical response at the EFU. On the other hand, no statistically significant differences were noticed for the duration of hepatitis, age, sex, use and duration of previous antiviral treatment (lamivudine or IFN). Also there was a strong correlation between decrease of HBV DNA and ALT normalization, all the patients with HBV DNA < 100,000 copies/mL and the end of follow-up registering normalized alanine aminotransferase level.

In conclusion, the results of our study demonstrate that peginterferon alfa 2a is safe and efficient in the treatment of HBeAg positive chronic hepatitis B patients, even with pre-therapeutic poor prognostic factors such as failure from previous therapies, high baseline viral load or genotype D infection.

Acknowledgements

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Conflicts of interests

None to declare.

References