Pegylated Interferon Alfa in HBeAg-positive Chronic Hepatitis B

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Gepegyleerde Interferon Alfa in HBeAg-positieve Chronische Hepatitis B

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	Prof. J.H.P. Wilson
	Prof.dr. H.W. Tilanus

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Chapter 1

Introduction

The hepatitis B virus: structure and morphology

The hepatitis B virus (HBV) belongs to the family of hepadna viridae and has a diameter of 42-47 nm[1]. The virus particle encloses a partially double-stranded DNA genome with a length of approximately 3200 base pairs. Within the viral DNA genome 4 open reading frames (ORFs) can be identified and are termed in analogy of their encoding proteins S (surface), C (core), P (Polymerase), and X (HBx protein). The ORF S contains 3 regions, the pre S1, pre S2 and S, which encode for the large, middle and small hepatitis B surface glycoproteins depending on the start of the transcription site, respectively. The ORF C is responsible for encoding the hepatitis B e antigen (HBeAg) and the core antigen (HBcAg). After the binding of the virus particle to the hepatocyte, the HBV viral genome is converted into covalently closed circular DNA (cccDNA) in the hepatocyte nucleus. The cccDNA forms the key template for pregenomic RNA in the HBV replication cycle (Figure 1) and acts as the reservoir for the HBV. In the hepatocyte cytoplasm, along with the core and polymerase proteins the pregenomic RNA is assembled to virus particles. Sequentially, the RNA is reversed transcribed into a HBV DNA minus strand, which is finally transcribed by a HBV DNA polymerase into the HBV DNA plus strand. The formed particle can either be excreted via the Golgi apparatus or recycled into the nucleus to form cccDNA[2].

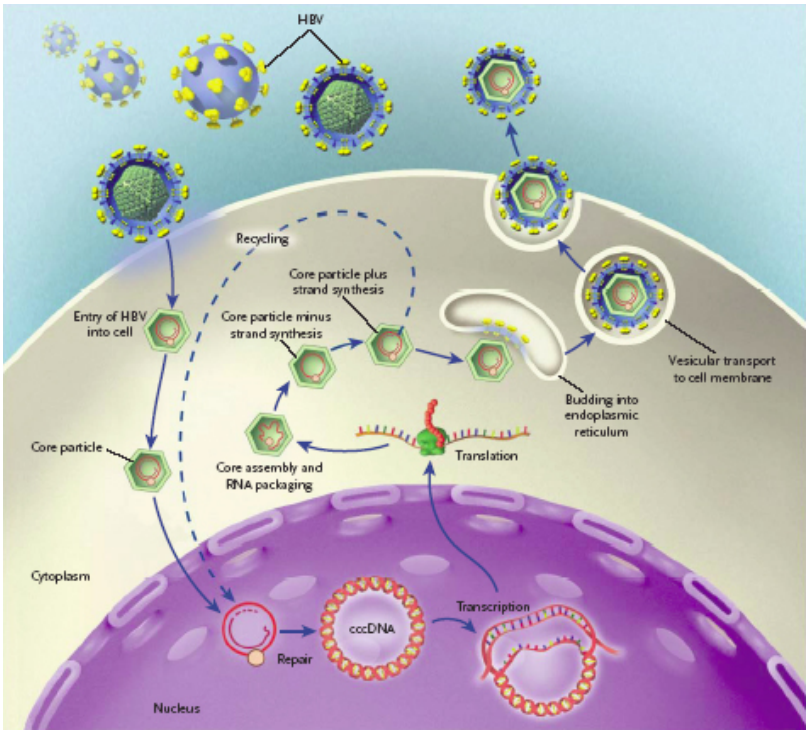

Figure 1. The hepatitis B virus replication cycle
(Ganem et al. N Engl J Med 2004)

Epidemiology

Worldwide, HBV infection is a common and important infection. Current estimates report infection by HBV in about 2 billion people. Of these, approximately 400 million are chronically infected by the HBV[3]. Chronic infection (serum HBsAg-positivity > 6 months) is associated with increased risk of developing liver cirrhosis and end-stage liver disease as decompensated cirrhosis and hepatocellular carcinoma (HCC)[4-6]. HCC is the most frequent malignant tumor of the liver and relates in 60-80% to chronic HBV[7]. It has been estimated that one million people die as the result of chronic HBV infection each year.

The mode of HBV transmission is through parenteral contact of body fluids such as transmission from mother to child during birth, sexual transmission, needle accidents or intravenous drug use. In endemic areas (>8% incidence HBV infection) the disease is often perinatally transmitted, whereas in areas with low incidence of HBV infection (<1%), intravenous drug use, tattooing and sexual transmission are the main routes of transmission[8]. Depending the age at time of exposure to HBV the risk for developing chronic HBV varies. During the first year of the newborn the chance for chronicity is approximately 90%, risk decreases to 30% between 1-5 years to approximately 2% above 5 years and adults[9].

HBV genotypes

As a result of variety in expression of the viral genome, the HBV is divided into 8 different genotypes, A-H[10, 11]. The HBV genotypes are also characterized by different geographical and demographical distribution. Genotype A is predominantly found in North-west Europe and North America, whereas genotypes B and C are mostly seen in Asian countries. Genotype D is most common in the Mediterranean area. Consequently, Caucasians harbour predominantly genotype A and D, and Asians almost exclusively the genotypes B and C[12, 13]. In the current studies evidence is building that HBV genotypes are becoming more important in the treatment of CHB. Erhardt and colleagues found that patients harbouring genotype A respond better to treatment with interferon than those with genotype D[14]. A study with peginterferon in HBeAg-positive chronic hepatitis B (CHB) showed higher response rates for genotypes A and B when compared to genotypes C and D, respectively[15]. Spontaneous HBeAg seroconversion seems to occur earlier in the natural course of the disease in patients harbouring genotype B than in genotype C which has also been associated with poorer clinical outcome[16, 17].

Treatment of Chronic Hepatitis B

The available therapeutic options for the treatment of chronic HBV are divided into two types of treatment. One group embraces the nucleoside or nucleotide analogues (NA's), the other group the interferons.

To date, lamivudine, adefovir and entecavir are the approved NA's for the treatment of chronic HBV. The NA's are capable of inhibiting the HBV polymerase and thereby the HBV replication. Lamivudine was the first NA approved for the treatment of chronic hepatitis B. It is a nucleoside analogue of deoxycytidine, the enantiomer of 2'-deoxy-3'-thicytidine. One year of treatment with lamivudine leads to HBeAg seroconversion in 16-18% of patients. Lamivudine significantly reduces HBV DNA levels, normalizes serum ALT levels and improves liver histology[18-20]. Long-term therapy with lamivudine is well tolerated with a minimum of side effects, but its efficacy is limited due to the emergence of therapy resistant strains[20, 21]. The viral resistance to lamivudine is caused by a point mutation in the YMDD motif of the HBV polymerase gene of the virus which significantly reduces the efficacy of lamivudine. These lamivudine resistant strains are found in up to 70% after 4-years treatment. Cessation of therapy is frequently accompanied by reoccurrence of HBV replication[22, 23].

Adefovir dipivoxil, an analogue of adenosine monophosphate was the second NA approved for the treatment of CHB. A large trial showed 12% HBeAg seroconversion, suppression of HBV DNA levels < 400 copies/mL in 21%, and histologic improvement in 53% after 48 weeks of treatment with adefovir. Resistance to adefovir was found in 3% of patients after 48 weeks treatment[24]. Entecavir, which has recently been approved for the treatment of CHB is a guanosine analogue and capable of stronger suppression of HBV DNA levels compared to lamivudine[25]. A 48-week treatment course with entecavir showed HBeAg seroconversion in 21%, suppression of HBV DNA levels < 400 copies/mL in 67%, and histologic improvement in 72% of patients. After 48 weeks no viral resistance to entecavir was detected.

Interferons act by their capacity of suppressing cell proliferation, enhancing phagocytic activity of macrophages, improved cytotoxicity of lymphocytes against target cells and inhibiting the viral replication[26, 27]. A meta-analysis of 498 patients showed that 3-6 months of IFN induced HBeAg loss in 33%, undetectable HBV DNA levels (by hybridization asssy) in 37% and HBsAg loss in 8% of patients. Loss of HBsAg is closely related to improved long-term histological and virological outcome as well as decreased risk of hepatic decompensation and hepatocellular carcinoma[7, 16, 28], and therefore regarded as the ultimate purpose of therapy for chronic hepatitis B. In contrast to IFN, the treatment with NA does not lead to enhanced HBsAg loss compared to spontaneous HBsAg seroconversion.

In the past few years, pegylated forms of IFN's have been introduced in the treatment of CHB. Pegylation embraces the attachment of a polyethylene glycol (Peg) to the IFN molecule which increases the half-life of the drug in the serum[29]. This allows dosing of peginterferon

once a week instead of three times per week for conventional IFN. Pegylated interferons have been reported to be more effective with a similar safety profile than conventional IFN in patients with chronic hepatitis C[30, 31]. Recently, two large trials demonstrated that peginterferon alfa-2a and peginterferon alfa-2b lead to HBeAg seroconversion in 32% and 29 % of HBeAg-positive CHB, respectively[15, 32]. Janssen et al. also demonstrated that higher baseline ALT levels, lower baseline HBV DNA levels, and HBV genotype were positive independent predictors for HBeAg seroconversion. The current available therapies are effective in approximately one-third of HBeAg-positive CHB patients. In order to enhance response rates combination therapy of immune stimulating combined with virus suppressive medication have been investigated, but the addition of lamivudine to conventional IFN or peginterferon did not lead to increased response rates compared to monotherapy with IFN or peginterferon[15, 32, 33]. The optimal treatment for the growing population of difficult-to-treat patients needs further investigation.

AIMS OF THE STUDY

To investigate the efficacy and adverse effects of peginterferon therapy alone or combined with lamivudine in HBeAg-positive CHB

To investigate the efficacy of peginterferon therapy in previous non-responders to conventional IFN and or lamivudine

To investigate the role of HBV genotype in response to peginterferon therapy

REFERENCES

1. Lau JY, Wright TL. Molecular virology and pathogenesis of hepatitis B. Lancet. 1993 Nov 27;342(8883):1335-40.
2. Ganem D, Prince AM. Hepatitis B virus infection--natural history and clinical consequences. N Engl J Med. 2004 Mar 11;350(11):1118-29.
3. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology. 2001 Dec;34(6):1225-41.
4. Di Marco V, Lo Iacono O, Camma C, et al. The long-term course of chronic hepatitis B. Hepatology. 1999 Jul;30(1):257-64.
5. Liaw YF, Tai DI, Chu CM, et al. The development of cirrhosis in patients with chronic type B hepatitis: a prospective study. Hepatology. 1988 May-Jun;8(3):493-6.
6. Lee WM. Hepatitis B virus infection. N Engl J Med. 1997 Dec 11;337(24):1733-45.
7. Beasley RP. Hepatitis B virus. The major etiology of hepatocellular carcinoma. Cancer. 1988 May 15;61(10):1942-56.
8. de Franchis R, Hadengue A, Lau G, et al. EASL International Consensus Conference on Hepatitis B. 13-14 September, 2002 Geneva, Switzerland. Consensus statement (long version). J Hepatol. 2003;39 Suppl 1:S3-25.
9. Hyams KC. Risks of chronicity following acute hepatitis B virus infection: a review. Clin Infect Dis. 1995 Apr;20(4):992-1000.
10. Norder H, Courouce AM, Magnius LO. Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. Virology. 1994 Feb;198(2):489-503.
11. Stuyver L, De Gendt S, Van Geyt C, et al. A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. J Gen Virol. 2000;81 Pt 1:67-74.
12. Lindh M, Andersson AS, Gusdal A. Genotypes, nt 1858 variants, and geographic origin of hepatitis B virus--large-scale analysis using a new genotyping method. J Infect Dis. 1997 Jun;175(6):1285-93.
13. Magnius LO, Norder H. Subtypes, genotypes and molecular epidemiology of the hepatitis B virus as reflected by sequence variability of the S-gene. Intervirology. 1995;38(1-2):24-34.
14. Erhardt A, Reineke U, Blondin D, et al. Mutations of the core promoter and response to interferon treatment in chronic replicative hepatitis B. Hepatology. 2000 Mar;31(3):716-25.
15. Janssen HL, van Zonneveld M, Senturk H, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet. 2005 Jan 8;365(9454):123-9.
16. Yuen MF, Wong DK, Sablon E, et al. HBsAg seroclearance in chronic hepatitis B in the Chinese: virological, histological, and clinical aspects. Hepatology. 2004 Jun;39(6):1694-701.

17. Kao JH, Chen PJ, Lai MY, et al. Genotypes and clinical phenotypes of hepatitis B virus in patients with chronic hepatitis B virus infection. J Clin Microbiol. 2002 Apr;40(4):1207-9.

18. Dienstag JL, Schiff ER, Wright TL, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med. 1999 Oct 21;341(17):1256-63.

19. Lai CL, Chien RN, Leung NW, et al. A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group [see comments]. N Engl J Med. 1998;339(2):61-8.

20. Lau DT, Khokhar MF, Doo E, et al. Long-term therapy of chronic hepatitis B with lamivudine. Hepatology. 2000 Oct;32(4 Pt 1):828-34.

21. Lok AS, Lai CL, Leung N, et al. Long-term safety of lamivudine treatment in patients with chronic hepatitis B. Gastroenterology. 2003 Dec;125(6):1714-22.

22. van Nunen AB, Hansen BE, Suh DJ, et al. Durability of HBeAg seroconversion following antiviral therapy for chronic hepatitis B: relation to type of therapy and pretreatment serum hepatitis B virus DNA and alanine aminotransferase. Gut. 2003 Mar;52(3):420-4.

23. Dienstag JL, Cianciara J, Karayalcin S, et al. Durability of serologic response after lamivudine treatment of chronic hepatitis B. Hepatology. 2003 Apr;37(4):748-55.

24. Marcellin P, Chang TT, S.G. L. Long-term efficacy and safety of adefovir dipivoxil (ADV) in HBeAg+ chronic hepatitis patients: increasing serology, virologic, and biochemical response over time. Hepatology. 2004;40 Suppl 1:665A.

25. Chang TT, Gish RG, de Man R, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med. 2006 Mar 9;354(10):1001-10.

26. Foster GR. Interferons in host defense. Semin Liver Dis. 1997;17(4):287-95.

27. Thomas H. Pathogenesis of chronic active hepatitis B. J Gastroenterol Hepatol. 1991;6 Suppl 1:4-6.

28. Yang HI, Lu SN, Liaw YF, et al. Hepatitis B e antigen and the risk of hepatocellular carcinoma. N Engl J Med. 2002 Jul 18;347(3):168-74.

29. Baker DE. Pegylated interferons. Rev Gastroenterol Disord. 2001;1(2):87-99.

30. Lindsay KL, Trepo C, Heintges T, et al. A randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. Hepatology. 2001 Aug;34(2):395-403.

31. Russo MW, Fried MW. Side effects of therapy for chronic hepatitis C. Gastroenterology. 2003 May;124(6):1711-9.

32. Lau GK, Piratvisuth T, Luo KX, et al. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med. 2005 Jun 30;352(26):2682-95.

33. Schalm SW, Heathcote J, Cianciara J, et al. Lamivudine and alpha interferon combination treatment of patients with chronic hepatitis B infection: a randomised trial [see comments]. Gut. 2000;46(4):562-8.

Chapter 2

The safety of pegylated interferon alfa-2b in the treatment of chronic hepatitis B: predictive factors for dose reduction and treatment discontinuation

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ABSTRACT

Background: Treatment with interferon-alfa has been shown to be effective in one-third of hepatitis B e antigen-positive chronic hepatitis B patients, but is clinically associated with relevant adverse events.

Aim: To investigate the safety of peginterferon alfa-2b in 300 hepatitis B e antigen-positive patients with compensated liver disease.

Methods: Patients were treated with peginterferon alfa-2b for 52 weeks combined with either lamivudine 100 mg/day or placebo. Peginterferon alfa-2b was administered for 100 µg once a week for 32 weeks; thereafter, the dose was reduced to 50 µg once a week. Adverse events and their effect on study medication were reported at monthly visits in a standardized way.

Results: The most frequently reported side-effects were flu-like syndrome (68%), headache (40%), fatigue (39%), myalgia (29%) and local reaction at the injection site (29%). These symptoms typically occurred within the first month of therapy and subsided during the course of therapy. Neutropenia and thrombocytopenia induced by peginterferon alfa-2b increased the risk of infections and bleeding complications, but these complications were rare and mild. The frequency of all side-effects was not different between patients treated with peginterferon alfa-2b combined with lamivudine or placebo. In 69 (22%) patients the dose of peginterferon alfa-2b was reduced prematurely. Of these dose reductions, 36 (52%) were because of neutropenia. Therapy was discontinued in 28 (8%) patients. The most frequent reasons for early discontinuation were psychiatric side effects (depression, psychosis) and flu-like symptoms. Multivariate Cox regression analysis showed that low neutrophil count at baseline and cirrhosis were independent predictors of dose reduction or therapy discontinuation.

Conclusion: We conclude that in patients with chronic hepatitis B and compensated liver disease prolonged peginterferon alfa-2b therapy is safe, and that pre-existent cirrhosis and neutropenia are the most important predictors of dose reduction or early treatment discontinuation.

INTRODUCTION

An estimated 400 million people are chronically infected with hepatitis B virus (HBV). Chronic hepatitis B (CHB) is the single most common cause of liver cirrhosis and hepatocellular carcinoma (HCC) worldwide[1]. Interferon-alpha (IFN) is effective in about one-third of the patients[2]. Reported HBeAg seroconversion rates range from 15 to 37%[3–5], depending on baseline characteristics such as alanine aminotransferase (ALT) levels and viral load. Response to IFN therapy was shown to result in sustained clearance of hepatitis B e antigen (HBeAg), HBV-DNA and normalization of aminotransferase levels[6–8]. Therapy with IFN is associated with considerable side-effects. Most frequently reported side-effects are flu-like syndrome, fatigue, headache and myalgia. Other clinically relevant side effects, such as depression, anorexia and insomnia, occur less frequently. Cases of suicide attempts during IFN therapy have been reported[9–11]. To improve response rates and possibly reduce the number of side effects, newer forms of IFN have been developed. 'Pegylated' forms of IFN (with a polyethylene glycolmoiety attached to it) have an improved pharmacokinetic profile with a prolonged half-life time[9]. Pegylated interferons have been reported to be safe and more effective than conventional IFN in patients with chronic hepatitis C[12,13].
Until now, the safety data of these pegylated forms of IFN in the treatment of CHB are limited to the study of Cooksley et al.[14], who investigated the safety of peginterferon alfa-2a in CHB patients. In this study, we assessed the safety of peginterferon alfa-2b (Peg-IFN α-2b) alone or in combination with lamivudine in HBeAg-positive CHB.

MATERIALS AND METHODS

Patients

In a randomized multicentre trial reported previously[15], 307 HBeAg-positive CHB patients with compensated liver disease were treated with Peg-IFN α-2b in combination with either lamivudine or placebo. Inclusion criteria were hepatitis B surface antigen (HBsAg)-positivity for at least 6 months, age ³ 16 years, ALT at least twice the upper limit of normal (ULN) and HBeAg positive on two occasions within 8 weeks prior to randomization. Patients were excluded if they had been treated with antiviral medication in the previous 6 months or any investigational drug within 30 days of entry in this protocol, were coinfected with hepatitis C, hepatitis D or human immunodeficiency virus (HIV), had alcoholic hepatitis or other causes of liver disease, had preexistent leucopenia or thrombocytopenia [white blood cell count (WBC) $\leq$ 3000/mm^3, neutrophils $\leq$ 1800/mm^3, platelets $\leq$ 100 000/mm^3], had decompensated liver disease (prothombin time prolonged by > 3 seconds, serum albumin < 35 g/L, ascites, encephalopathy, history of variceal bleeding) or had hypothroidism or hyperthyroidism. Patients were also excluded in case of pregnancy, inadequate contraception,

any significant medical illness potentially interfering with the study or any contraindication specified for IFN. Ethics committees of the participating centres approved of the protocol and all patients provided written informed consent.

Study design

In this double-blinded trial, eligible patients were randomized to one of two treatment regimens (Figure 1). All patients received Peg-IFN α-2b for 52 weeks. Patients were treated with a 100 µg dose of Peg-IFN α-2b once a week until week 32, whereafter it was reduced to 50 µg once a week. In addition, patients received either placebo or 100 mg lamivudine orally. Patients were examined every 4 weeks during treatment and during the 6-month post-treatment follow-up. At visits, routine physical examination was performed and blood samples were obtained for haematological and biochemical screening [haemoglobin, WBC, neutrophils, platelets, ALT and aspartate aminotransferase (AST)]. All adverse events were reported by the treating physician on standard case-record forms and verified by the trialcoordinating centre. All participating investigators were instructed, received a protocol and were monitored every 3 months in order to ensure uniform scoring of side effects. Adverse events were graded according to the WHO recommendations for grading of acute and subacute toxicities[16], and reported as mild, moderate, severe or life-threatening. The reported adverse events were also assessed in their relation to therapy by the treating physician and reported as unrelated, possibly related, probably related or related to therapy. Effect on study medication was scored as none, dose reduction or treatment discontinuation. Serious adverse events (SAE) were defined as events resulting in death, events that are life-threatening, require or prolong in-patient hospitalization, as well as events which result in persistent or significant disability or incapacity, pregnancy, any congenital anomaly, cancer, or drug overdose. Hepatitis flares were defined as an increase in serum ALT to at least three times the baseline level. Psychiatric side effects included mood changes, irritability and depression. Autoimmune phenomena are not reported because they were not systematically investigated with, e.g. longitudinal assessment of auto-antibodies.

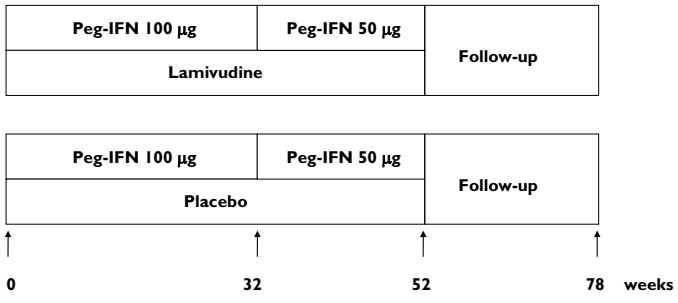

Figure 1. Treatment Schedule

Guidelines on discontinuation of therapy in case of flares were provided to each investigator before the start of the therapy. The decision to stop therapy in patients with flares and signs of diminished liver function was left at the responsibility of the participating physician who treated the patient.

Statistical analysis

Kaplan–Meier analysis was used to assess time until dose reduction or premature discontinuation of therapy. For univariate analysis, the following factors were considered: age, gender, race, mode of transmission, baseline levels of AST, ALT, bilirubin, neutrophils, platelets and HBV-DNA, previous therapy with IFN or lamivudine and presence of cirrhosis. To assess which variables independently predicted dose reduction or early discontinuation of therapy, variables that were significant or approximated significance in the univariate analysis ($p < 0.2$) were included in a multivariate analysis. Cox regression was used for multivariate analysis. Chi-square analysis was carried out to compare frequencies of adverse events. All statistical analyses were performed in SPSS version 10 (SPSS Inc, Chicago, IL, USA).

RESULTS

Baseline characteristics

A total of 307 patients were randomized to treatment with Peg-IFN α-2b and either lamivudine or placebo (ratio 1:1). The analysis of efficacy, that was reported previously, was performed for 266 patients, and excluded patients who never started treatment (n=7), were HBeAg-negative at the start of treatment (n=10), and patients from a single centre where source data could not be verified (n=24)[15]. Because safety data were available and could be verified for all patients, the 300 patients who received at least one dose of medication were included in the present safety analysis. The baseline characteristics of these patients are shown in Table 1. A total of 228 males (76%) and 72 females were treated with Peg-IFN α-2b in combination with either lamivudine (n=148) or placebo (n=152). The mean age of the patients was 35 years (range 16–72). The majority of patients (76%) were Caucasians. For 230 patients, pre-treatment biopsies were available and of sufficient quality to be evaluated. Of these patients, 26 (11%) had cirrhosis at liver biopsy. Among the 300 patients, 71 patients (24%) had been treated with IFN, and 45 (16%) with lamivudine prior to entry in this study.

Table 1. Patient characteristics at baseline

Characteristics	(n = 300)
Age (yrs)	35 ± 13
Gender, n (%)	
Male	228 (76)
Female	72 (24)
Weight (kg) *	73 ± 14
Race, n (%)	
Caucasian	228 (76)
Asian/ Other	72 (24)
Mode of transmission, n (%)	
Vertical	66 (22)
(Homo) sexual	31 (10)
Parenteral	27 (9)
Transfusion associated	8 (3)
Unknown	173 (56)
Previous IFN therapy, n (%)	71 (24)
Previous lamivudine therapy, n (%)	45 (16)
Histological diagnosis (%)	
Cirrhosis	26 (11)
No cirrhosis	204 (89)
ALT (x ULN)*	4.3 ± 3.5
Platelets (10^9/L)	204 ± 60
Neutrophils	3.4 ± 1.3
Log HBV DNA*	9.0 ± 1.1

IFN, interferon; ALT, alanine aminotransferase; HBV, hepatitis B virus; ULN, upper limit of normal. * Mean ± s.d.

Side effects

All treated patients reported one or more of the known side-effects of IFN. The frequency of all side-effects was not significantly different between the Peg-IFN α-2b/placebo group and the Peg-IFN α-2b/lamivudine group. Moreover, there was no difference in occurrence of SAE and in the need for dose reduction or premature treatment discontinuation between the treatment groups. Therefore, we combined the data of the two groups for all analyses. The most frequently reported adverse events were flu-like syndrome (68%), headache (40%), fatigue (39%), myalgia (29%) and local reaction at the injection site (29%) (Table 2).

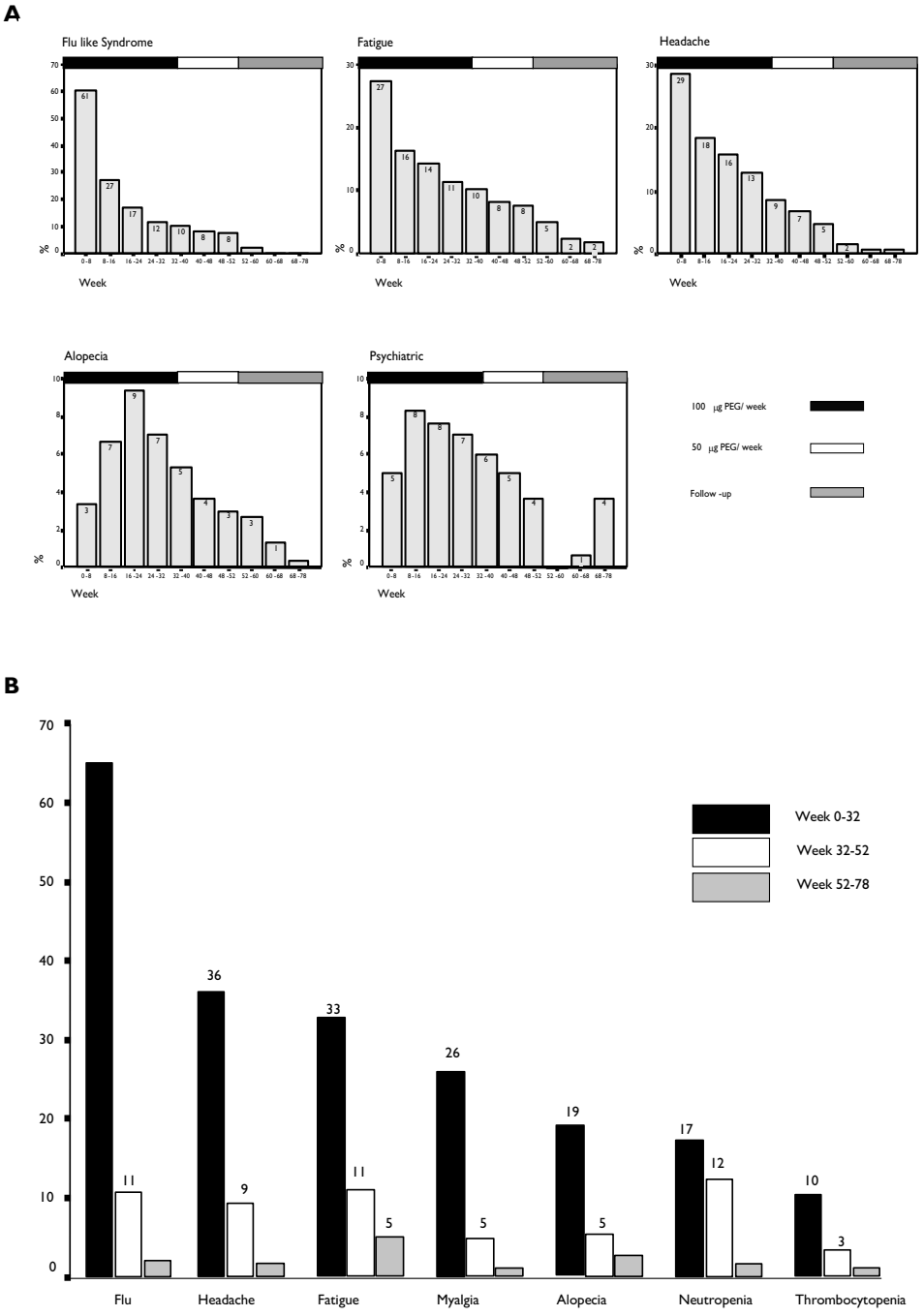

Figure 2. (A) Course of frequent adverse events during therapy and follow-up. (B)Adverse events: Frequency of events in relation to peginterferon alfa-2b dose.

These symptoms typically occurred within the first month of therapy and mostly subsided during the course of therapy. Alopecia and psychiatric symptoms (including depression (n=26) and mood changes or irritability without depression (n=33)) occurred later in the course of therapy (Figure 2). Transient visual impairment was recorded in five patients (2%), but were not a reason for dose reduction or early treatment discontinuation. During therapy, leucopenia (< 3.0 x10^9 U/L, grade II), neutropenia (< 1.5 x10^9 U/L, grade II) and thrombocytopenia (< 75 x10^9 U/L, grade II) occurred in 42, 22 and 12% of the patients, respectively (Table 2). All adverse events were reported less frequently after dose reduction and were completely reversed after the end of therapy (Figure 2).

Hepatitis flares. A total of 71 hepatitis flares were reported, occurring during therapy in 31 patients and after the end of therapy in 40 patients. Flares occurred in 25% (37/148) of patients in the Peg-IFN α-2b/lamivudine group and in 22% (34/152) of patients in the Peg-IFN α-2b/placebo group (p = 0.5). The frequency of on- and posttreatment flares was not different between groups. Two flares led to discontinuation of therapy. One patient had signs of diminished liver function in the course of a flare. This patient had elevated bilirubin levels (62 µmol/L), which resolved after normalization of ALT levels, but no other signs of hepatic decompensation. None of the hepatitis flares resulted in sustained decompensated liver disease or death.

Table 2. Frequencies of adverse events

Adverse event	Freq %
Flu like Syndrome	68
Headache	40
Fatigue	39
Myalgia	29
Local reaction	29
Alopecia	22
Weight loss (> 10 %)	19
Psychiatric*	20
Abdominal pain	15
Haematologic events	
Leucopenia (< 3.0 x 10^9 u/L)	42
Neutropenia (< 1.5 x 10^9 u/L)	22
Thrombocytopenia (< 75 x 10^9 u/L)	12

* Includes depression, mood changes, irritability.

Serious adverse events. During the study, 33 SAE were reported. Of these 17 were judged as probably related to therapy. Four patients had a serious hepatitis flare, and three developed severe depression. Neutropenia (gradeIII or IV) was reported in three cases. Other SAE that were probably related to therapy were syncope, seizures, psychosis, pancreatitis, diarrhoea, anxiety and dizziness (resulting in a fall and a head wound). In 14 patients, the serious adverse event led to early discontinuation of therapy [depression (n=4), flare (n=2), neutropenia, seizures, pneumonia, psychosis, pancreatitis, severe diarrhoea, allergic reaction with hypotension and pregnancy (all n=1)].

Bacterial infections and bleeding complications. Bacterial infections occurred in 12 patients (4%). Nine of these 12 patients had neutropenia of at least grade II (< 1.5 $x10^9$/L) (Table 3). The risk of infection increased with an increasing severity of neutropenia. Patients with neutrophil counts below 1.5 $x10^9$/L had a significantly increased risk of infection compared with patients with higher neutrophil counts (13.6% vs. 1.3%; $p < 0.001$). Infections included urinary tract infection (n=4), gastroenteritis (n=2), tonsillitis (n=2), gingivitis (n=2), appendicitis (n=1) and pneumonia (n=1). Overall, three severe bacterial infections were reported. In two of these cases (pneumonia and appendicitis) the neutrophil count never dropped below 1.5 $x10^9$/L. The third patient had a Salmonella gastroenteritis while his neutrophil count was 0.7 $x10^9$/L. In four cases [pneumonia, appendicitis and gastroenteritis (n= 2)] the infection necessitated hospital admission. All infections resolved without lasting complications. The risk of bleeding complications was increased in patients with thrombocytopenia, especially of grade III (< 50 $x10^9$/L) or IV. Reported bleeding complications in these patients were epistaxis (n=2), gastrointestinal bleeding, subcutaneous bleeding and combined ecchymosis plus epistaxis (Table 4a). Cirrhotic patients (n= 25) had a significantly higher risk of developing thrombocytopenia than non-cirrhotic patients (54% vs. 8%, $p < 0.001$). Three of the cirrhotic patients (with thrombocytopenia grade II and III) had bleeding complications (Table 4b). The reported bleeding complications were not life-threatening and did not necessitate blood transfusions.

Table 3. Bacterial infections in patients with low neutrophil count

Neutrophils ($x10^9$ U/L)	n	Bacterial Infections (%)
> 1.5	234	3 (1.3)
1.0- 1.5	42	7 (16.6)
0.5-1.0	15	1 (6.6)
< 0.5	9	1(11.1)

Reported bacterial infections: urinary tract infection (n=4), gastroenteritis (n= 2), tonsillitis (n= 2), gingivitis (n=2), pneumonia (n=1), appendicitis (n=1).

Table 4. Bleeding complications according to level of thrombopenia (A) and cirrhosis (B) in patients with low platelets

A

Platelets (x 10^9 U/L)	n	Bleeding Complications (%)
> 100	222	6(2.7)
75-100	41	2 (4.9)
50-75	28	1 (3.6)
< 50	9	2(22.2)

B

	Cirrhosis (n = 26)		No Cirrhosis (n = 204)	
Platelets (U/L)	n	Bleeding complications n (%)	n	Bleeding complications n (%)
> 75 x 10^9	12	0 (0)	188	4 (2)
< 75 x 10^9	14	3 (21)	16	0 (0)

Reported bleeding complications in patients with platelets < 100 x10^9 U/L: epistaxis n=2), gastrointestinal bleeding (n=1), subcutaneous bleeding (n=1), ecchymosis and epistaxis (n=1).

Dose reduction and discontinuation of therapy

In 69 patients (23%), the dose of Peg-IFN α-2b was reduced prematurely (Table 5). Of the dose reductions, 37 (54%) occurred in the Peg-IFN α-2b/lamivudine group and 32 (47%) in the Peg-IFN α-2b/placebo group. The dose of the blinded drug (lamivudine/placebo) was not reduced in any of the patients. The main reasons for dose reduction were neutropenia (52%), thrombocytopenia (10%), flu-like syndrome (10%) and combined haematological abnormalities (8%). In four patients (6%), dose reduction was necessary because of psychiatric symptoms and in seven patients because of flu-like-syndrome. Fifty percent of the dose reductions occurred within the first 10 weeks. Thereafter, the number of dose reductions decreased. Only two dose reductions were reported after week 32.

Peg-IFN α-2b therapy was discontinued prematurely in 28 patients (9%). In 24 patients, the blinded drug (lamivudine/placebo) was also discontinued. In 10 patients, therapy discontinuation was due to psychiatric side-effects. The second clinically important reason for discontinuation was flu-like syndrome (n=3). Other reasons for early discontinuation were acute pancreatitis, flare, decompensated liver disease and seizures (Table 5). Discontinuation of therapy was reported more frequently before the scheduled dose reduction of Peg-IFN α-2b at week 32. In univariate analysis, a low neutrophil count (< 3 x10^9/L) at baseline and presence of liver cirrhosis were associated with an increased risk of dose reduction or discontinuation of therapy (Table 6). Cox regression analysis, including all variables with a p-value < 0.2 in the univariate analysis, showed that both low neutrophil count (hazard ratio 1.7, p = 0.03) and cirrhosis (hazard ratio 2.5, p = 0.001) remained the only independent predictors of dose reduction or discontinuation of therapy.

Table 5. Reasons for dose reduction and early discontinuation

Dose reduction	n (%)	Early discontinuation	n (%)
Neutropenia	36 (52)	Psychiatric	10 (36)
Thrombocytopenia	7 (10)	Flu like syndrome	3 (11)
Leucopenia	2 (3)	Patient lost to follow-up	4 (14)
Combined hematological	6 (8)	Anemia	1 (4)
Flu like syndrome	7 (10)	Neutropenia	1 (4)
Psychiatric	4 (6)	Thrombocytopenia	1 (4)
Fatigue	2 (3)	Flare	1 (4)
Local reaction	1 (1)	Seizures	1 (4)
Anorexia	1 (1)	Acute pancreatitis	1 (4)
Myalgia	1 (1)	Decompensated liver disease	1 (4)
Other	2 (3)	Pneumonia	1 (4)
		Other	3 (11)
Total	69	*Total*	28

DISCUSSION

The side-effects of conventional IFN therapy have been well documented in patients with chronic hepatitis B and C[8, 12, 14, 17, 18]. The most common side effects include influenza-like symptoms, fatigue, gastrointestinal symptoms (nausea, anorexia, weight loss), alopecia and neuropsychiatric symptoms (irritability, depression, insomnia). IFN also causes mild bone marrow depression with a temporary decrease in neutrophil, leucocyte and platelet counts. These side effects have an impact on the quality of life, and can lead to dose reduction or treatment discontinuation. This report is the first on safety of Peg-IFN α-2b in patients with CHB. All patients reported one or more of the known adverse events of standard IFN. The adverse events reported in our patients are largely those expected of standard IFN, and no new events were reported. The frequency of the most common adverse events – flu-like syndrome (66%), headache (39%), fatigue (37%) and myalgia (28%) – was comparable with those previously reported in CHB patients treated with standard IFN in a dose of 4.5 MU14 or 10 MU t.i.w.[17] for 16–32 weeks. Obviously, these comparisons with historical controls must be interpreted with caution, as the treatment duration in our study was longer and patient populations and therapy doses differ between studies.

Pegylated interferons have been reported to be safe and more effective in patients with chronic hepatitis C[8, 11, 12, 18, 19]. In hepatitis C patients, the side-effect profile is the same as that of standard IFN, with some difference in frequencies between different doses and formulations. Haematological abnormalities (especially neutropenia) occur more frequently

Table 6. Univariate analysis of association of baseline factors with dose modifications (dose reduction or discontinuation of therapy)

Variable	Risk of dose modifications (%)	P-value
Age		
<35	27	0.19
≥35	34	
Sex		
Male	29	0.27
Female	35	
Weight		
<75 kg	34	0.11
≥75 kg	25	
Race		
Caucasian	28	0.07
Asian/ Other	37	
ALT		
< 4x ULN	29	0.47
≥ 4x ULN	32	
Log HBV DNA		
< 9	36	0.06
≥ 9	26	
Bilirubin		
< 11	28	0.57
≥ 11	31	
Platelets (x 10^9/L)		
< 200	34	0.13
≥ 200	25	
Neutrophils (x 10^9/L)		
<3	39	0.003
≥3	24	
Cirrhosis		
No	29	0.0005
Yes	62	
Previous IFN therapy		
Yes	34	0.34
No	28	
Previous lamivudine therapy		
Yes	38	0.15
No	27	

in chronic hepatitis C patients treated with pegylated interferons (Peg-IFN α-2b or peginterferon alfa-2a) than with standard IFN. The rate of dose reductions (22%) and therapy discontinuations (9%) in our study is comparable with the frequencies reported with pegylated interferons in patients with chronic hepatitis C[8, 12, 13, 20] and with 24 weeks of

peginterferon alfa-2a in patients with CHB[14]. The proportion of patients withdrawn from therapy with pegylated interferons is similar to that of conventional IFN, but, when compared with standard IFN, the proportion of patients requiring dose reductions is higher with Peg-IFN α-2b in most hepatitis C studies[8, 11, 12, 18]. In CHB patients, the frequency of dose reduction with peginterferon alfa-2a was higher than with conventional IFN (22–30% vs. 10%)[14]. The increased rate of dose reductions with pegylated interferons seems mainly because of the increased occurrence of neutropenia.

Until now it was unknown whether Peg-IFN α-2b-induced neutropenia and thrombocytopenia indeed lead to an increase of bacterial infections and bleeding complications respectively. In our study, neutropenia of < 1.5 $x10^9$/L significantly increased the risk of bacterial infections (Table 5). However, the number of bacterial infections was rather low and the infections were relatively mild. Only one serious infection (Salmonella gastroenteritis) occurred in a patient with neutropenia above grade I. The risk of bleeding complications was increased in patients with more severe thrombocytopenia, especially in patients with pre-existent cirrhosis and platelets dropping below 75 $x10^9$ U/L. However, bleeding complications were generally mild (epistaxis). Only one potentially life-threatening bleeding complication (bleeding duodenal ulcer) occurred in a patient with mild liver fibrosis.

We also investigated the course of side-effects and adherence to therapy over time. Side effects were most pronounced at the beginning of therapy and subsided over time. They were generally well tolerated, but flu-like symptoms, lethargy and depression in some cases necessitated dose reduction. Informing patients about the course of these adverse events and adequate treatment with paracetamol and specific serotonin reuptake inhibitors (SSRIs) may lead to an increased proportion of patients capable of completing the treatment. Neutropenia and thrombocytopenia are other frequent causes for dose reduction. Considering the mildness and rareness of complications of neutropenia in our patients, it might be worthwhile to investigate, in a randomized study, if it is safe to accept grade III neutropenia without dose reduction during Peg-IFN α-2b treatment for CHB. As, in our study particularly, cirrhotic patients were at an increased risk of thrombocytopenia and (minor) bleeding complications we recommend to monitor them closely and avoid a decrease in platelet count below 75 $x10^9$/L. In a previous study in 217 cirrhotic patients with chronic hepatitis C treated with peginterferon alfa-2a, despite substantial decreases in neutrophil and platelet counts, episodes of infection and bleeding were mild and the treatment was reported to be safe for this patient population[21].

In conclusion, prolonged treatment with Peg-IFN α-2b in patients with CHB and compensated liver disease is safe. Adding lamivudine to Peg-IFN α-2b did not affect Peg-IFN α-2b related side effects. Haematological abnormalities during Peg-IFN α-2b treatment led to an increased risk of minor infections and bleeding complications. Cirrhosis and low neutrophil count at baseline are independent predictors of dose reduction or therapy discontinuation. In our

opinion, one should closely monitor patients with cirrhosis when initiating Peg-IFN α-2b treatment.

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REFERENCES

1. Lee WM. Hepatitis B virus infection. N Engl J Med 1997; 337: 1733–45.
2. Wong DK, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. Ann Intern Med 1993; 119: 312–23.
3. Perrillo RP, Schiff ER, Davis GL, et al. A randomized, controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of chronic hepa titis B. The Hepatitis Interventional Therapy Group. N Engl J Med 1990; 323: 295–301.
4. Hoofnagle JH, Peters M, Mullen KD, et al. Randomized, controlled trial of recombinant human alpha-interferon in patients with chronic hepatitis B. Gastroenterology 1988; 95:1318–25.
5. Schalm SW, Heathcote J, Cianciara J, et al. Lamivudine and alpha interferon combi nation treatment of patients with chronic hepatitis B infection: a randomised trial. Gut 2000; 46: 562–8.
6. Niederau C, Heintges T, Lange S, et al. Long-term follow-up of HBeAg-positive patients treated with interferon alfa for chronic hepatitis B. N Engl J Med 1996; 334: 1422–7.
7. Lau DT, Everhart J, Kleiner DE, et al. Long-term follow-up of patients with chronic hepatitis B treated with interferon alfa. Gastroenterology 1997; 113: 1660–7.
8. Reddy KR, Wright TL, Pockros PJ, et al. Efficacy and safety of pegylated (40-kd) interferon alpha-2a compared with interferon alpha-2a in noncirrhotic patients with chronic hepatitis C. Hepatology 2001; 33: 433–8.
9. Baker DE. Pegylated interferons. Rev Gastroenterol Disord 2001; 1: 87–99.
10. Fattovich G. Natural course and prognosis of chronic hepatitis type B. Viral Hep Rev 1996; 2: 263–276.
11. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa- 2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002; 347: 975–82.
12. Lindsay KL, Trepo C, Heintges T, et al. A randomized, doubleblind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. Hepatology 2001; 34: 395–403.
13. Russo MW, Fried MW. Side effects of therapy for chronic hepatitis C. Gastroenterology 2003; 124: 1711–9.
14. Cooksley WG, Piratvisuth T, Lee SD, et al. Peginterferon alpha-2a (40 kDa): an advance in the treatment of hepatitis B e antigen-positive chronic hepatitis B. J Viral Hepat 2003; 10:298–305.
15. Janssen HL, van Zonneveld M, Senturk H, et al. Pegylated interferon alpha-2b alone or in combination with lamivudine as treatment for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005; 365: 123–9.
16. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer 1981; 47: 207–14.

17. Janssen HL, Gerken G, Carreno V, et al. Interferon alfa for chronic hepatitis B infection: increased efficacy of prolonged treatment. The European Concerted Action on Viral Hepatitis (EUROHEP). Hepatology 1999; 30: 238–43.

18. Zeuzem S, Feinman SV, Rasenack J, et al. Peginterferon alfa-2a in patients with chronic hepatitis C. N Engl J Med 2000;343: 1666–72.

19. Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001; 358: 958–65.

20. Fried MW. Side effects of therapy of hepatitis C and their management. Hepatology 2002; 36 (5 Suppl 1): S237–44.

21. Heathcote EJ, Shiffman ML, Cooksley WG, et al. Peginterferon alfa-2a in patients with chronic hepatitis C and cirrhosis. N Engl J Med 2000; 343: 1673–80.

Chapter 3

Flares in chronic hepatitis B patients induced by the host or the virus? Relation to treatment response during peginterferon α-2b therapy

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ABSTRACT

Background and aims: Flares are a well known phenomenon during antiviral treatment for chronic hepatitis B. Little is known about the effect of flares on response. We investigated the timing and characteristics of flares, in relation to treatment response (hepatitis B e antigen loss).

Patients: A total of 266 patients, participating in a global randomised controlled study, were assigned to 52 weeks of 100 µg peginterferon α-2b weekly, combined with either daily lamivudine 100 mg or placebo.

Results: Sixty seven patients (25%) exhibited 75 flares, with 38 (51%) flares in the combination therapy and 37 (49%) in the monotherapy groups. Overall, 30% of patients with and 38% of patients without a flare responded to therapy ($p = 0.25$). In 24 patients (36%) the flare was followed by a decrease in hepatitis B virus (HBV) DNA (host induced flare). In 25 patients (38%) the flare was preceded by an increase in HBV DNA (virus induced flare). In 17 patients (26%) the flare did not meet one of these criteria (indeterminate flare). Of patients with host induced flare, 58% responded whereas only 20% of patients with virus induced flares responded ($p = 0.008$). Hepatitis B surface antigen loss (n=8) was exclusively seen in patients experiencing a host induced flare. Multivariate logistic analysis showed that host induced flares was an independent predictor of response ($p = 0.043$).

Conclusion: Flares are not more common in responders than in non-responders to peginterferon α-2b therapy. Virus induced flares, which occur after an increase in HBV DNA level, and most probably are indicative for increased expression of viral antigens, did not lead to treatment response. In contrast, host-induced flares which were followed by a HBV DNA decrease were highly associated with treatment response.

INTRODUCTION

Approximately 400 million people worldwide are chronically infected with the hepatitis B virus (HBV). Chronic infection with HBV can lead to progression of liver diseases with increased risk of cirrhosis, liver failure, and hepatocellular carcinoma[1]. Currently, interferon á (IFN), lamivudine, and adefovir are the only registered drugs for treating chronic hepatitis B (CHB). During treatment with IFN and after withdrawal of lamivudine therapy, flares of inflammatory activity are a well-known phenomenon in CHB patients. Flares can be life threatening but have also been associated with virological response. IFN induced flares affect 25–40% of patients and have been attributed to the stimulatory effect of IFN, which is capable of increasing T cell cytolytic activity and natural killer cell function[2]. Typically, these flares are thought to occur in hepatitis B e antigen (HBeAg) positive patients during the second to third month of therapy, and may precede HBeAg seroconversion[2–5]. In our previous observation, flares during IFN were accompanied by an increased number of CD8+ specific T lymphocytes[6]. Lamivudine related flares are seen during treatment but they do not occur more often than in the natural course of CHB[3]. More important appear to be the flares found after withdrawal of lamivudine, which occur in approximately 10–20% of patients[3,7]. These flares are probably caused by reoccurrence of HBV replication, and have been associated with decompensation of liver disease. To clarify the role of flares during and after cessation of therapy, and to determine their relation with treatment response, we analysed 266 HBeAg-positive CHB patients who received pegylated (Peg)-IFN α-2b alone or in combination with lamivudine.

PATIENTS AND METHODS

Patients and study design

Data were extracted from a global multicentre randomised controlled trial comparing Peg-IFN α-2b combined with either lamivudine or placebo in CHB[8]. Patients were assigned in a 1:1 ratio to receive 100 µg Peg-IFN α-2b weekly with 100 mg lamivudine daily (combination therapy) or 100 mg Peg-IFN α-2b weekly with placebo (monotherapy). Duration of therapy was 52 weeks. After 32 weeks, the dose of Peg-IFN α-2b was halved to 50 µg per week. Post-treatment follow up lasted 26 weeks. Patients were eligible for treatment if they were 16 years of age or older, had been positive for hepatitis B surface antigen (HBsAg) for at least six months, had been HBeAg positive on two occasions within eight weeks prior to randomisation, and had two episodes of elevated serum alanine aminotransferase (ALT) levels (at least twice the upper limit of normal (ULN)) on two occasions within eight weeks prior to randomisation. Patients were excluded for the following reasons: treatment with antiviral medication within six months or any investigational drug within 30 days of entry to

the protocol, or presence of serum antibodies against hepatitis C virus, hepatitis D virus, or human immunodeficiency virus. Other exclusion criteria were: alcoholic hepatitis or other causes of liver disease; pre-existing leucopenia (white blood cell count ≤ 3000/mm^3), thrombocytopenia (platelets ≤ 100 000/mm^3), or granulocytopenia (granulocyts ≤ 1800/mm^3); decompensated liver disease (prothombin time prolonged by > 3 seconds, serum albumin <35 g/l, ascites, encephalopathy, history of variceal bleeding), or hypo- or hyperthyroidism. Patients were also excluded in the event of any contraindication specified for IFN. The ethics committee at the participating centres approved the protocol, and all patients provided written informed consent.

Monitoring

All patients were seen monthly during therapy and follow up. At each visit, patients attended the outpatient clinic for ALT measurement and other laboratory assessments.

Transaminases were assessed locally and expressed as xULN. In accordance with Honkoop and colleagues[7], a flare was defined as a threefold increase in serum ALT compared with baseline levels. The time point of the flare was defined as the time of the peak level of serum ALT. Multiple peak levels of thrice baseline serum ALT levels were considered as different flares if they were separated by at least two measurements of ALT. In addition to ALT, HBV DNA (detection limit 400 copies/mL, using inhouse Taqman PCR based on the Eurohep standard[9]) was assessed at the same time points. Other virological parameters, such as HBeAg (AxSYM; Abbott, Chicago, Illinois, USA) and HBsAg (AxSYM; Abbott) were assessed at baseline, and at weeks 32, 52 (end of treatment), and 78 (end of follow up). HBV genotype was assessed by Inno-Lipa assay (Innogenetics, Gent, Belgium). Response to therapy was defined as serum HBeAg loss at the end of follow up.

Statistical analysis

The chi-square or Fisher's exact test was used for categorical variables, and the Mann-Whitney U test was performed for continuous data. In order to determine independent predictors for the event flare, the baseline characteristics age, race, sex, mode of transmission, pre-existing cirrhosis, ALT, log HBV DNA, HBV genotype, and previous IFN were included in the univariate analysis. All tested variables with a p value < 0.15 were entered in the multivariate time dependent Cox regression analysis. In order to determine independent predictors for response within the flare population, we included the above mentioned baseline variables plus timing of the flare, peak value of ALT during exacerbation, and flare type (host induced versus virus induced) in a univariate and multivariate analysis. In the event of multiple flares, the first flare was analysed for response to therapy. All data were analysed using SPSS (version 10.1; SPSS Inc., Chicago, Illinois, USA). A p value of 0.05 was considered significant (all two tailed).

RESULTS

Flare versus non-flare

Among the 266 patients analysed, 75 flares were recorded in 67 patients (25%). Six patients experienced two flares and one patient three flares during treatment or follow up. Median time point of flare was at week 60 (range 4-78), and median peak of ALT during flares was 12.3 xULN (range 2.3- 60.0). Of the 75 flares, 35 (47%) occurred during treatment and 40 (53%) after treatment discontinuation. In three patients, of whom two had pre-existing cirrhosis, the flare was reported as a serious adverse event; in one patient this led to early cessation of treatment. One patient had signs of diminished liver function (bilirubin 62 µmol/l) during the flare episode which resolved after normalisation of ALT values. During the flare there were no other signs of hepatic decompensation. Characteristics of patients with and without flare are given in Table 1.

Table 1. Characteristics at baseline of 266 patients with or without a flare of chronic hepatitis B

Baseline	Flare N = 67	No Flare N = 199	P
Age*	34±12	35±13	0.804
ALT* (x ULN)	2.9±1.4	4.8±3.8	<0.001
Log HBV DNA*	9.1±1.1	9.1±0.9	0.764
Male (%)	52(78)	153(77)	0.947
Transmission (%)			
- vertical	18(27)	42(21)	
- (homo)sexual	7(10)	22(11)	0.132
- parenteral	8(12)	21(11)	
- transfusion	4(6)	3(2)	
- unknown	30(45)	111(56)	
Genotype (%)			
- A	19(28)	71(36)	
- B	7(10)	16(8)	0.738
- C	11(16)	28(14)	
- D	26(39)	77(39)	
Race (%)			
- Caucasian	47(70)	149(75)	0.432
- Asian	13(19)	39(20)	
Cirrhosis (%)	10(19)	14(7)	0.060
Previous lamivudine (%)	8(12)	31(16)	0.459
Previous Interferon (%)	12(18)	47(24)	0.311
Mono-therapy (%)	32(48)	104(52)	
Combination therapy (%)	35(52)	95(48)	0.566

Except for ALT and cirrhosis, all variables at baseline were comparable in the flare and non-flare groups. Ten patients (19%) in the flare group and 14 patients (9%) in the non-flare group had pre-existing cirrhosis (p = 0.06). Preexisting cirrhosis (p = 0.046; relative risk 2.0 (95% confidence interval (CI) 1.0-4.0)) and lower ALT at baseline (p = 0.0001; relative risk 1.4 (95% CI 1.2-1.6)) were also the only two independent predictors for experiencing a flare during therapy or follow up. Among the 75 flares, we recorded 37 (49%) in the monotherapy group (35 patients) and 38 (51%) in the combination therapy group (32 patients). Baseline characteristics and response to therapy were not significantly different between patients with a flare undergoing monotherapy or combination therapy (Table 2). In five patients who exhibited a flare during or after treatment with a combination of Peg-IFN α-2b and lamivudine, a YMDD mutant was detected. None of the flares was related to emergence of a YMDD mutant.

Table 2. Characteristics of patients who had a flare, according to therapy

	Peg-IFN α-2b Placebo N = 32 (48%)	Peg-IFN α-2b Lamivudine N = 35 (52%)	P- value
Age*	36 ± 13.1	33 ± 10.8	0.44
Male (%)	24 (75)	28 (80)	0.77
Race (%)			
Caucasian	21 (66)	26 (74)	0.73
Asian/Mongoloid	7 (22)	6 (17)	
ALT* (x ULN)	2.9 ± 1.3	2.9 ± 1.5	0.94
Log HBV DNA*	8.9 ± 1.3	9.2 ± 0.9	0.25
Genotype (%)			
A	9 (28)	10 (29)	
B	2 (6)	5 (14)	0.46
C	7 (22)	4 (11)	
D	11 (34)	15 (43)	
Pre-existing Cirrhosis (%)	5 (16)	5 (14)	0.99
Dose reduction (%)	11 (34)	13 (37)	0.81
Discontinuation of treatment (%)	4 (13)	5 (14)	0.83
Flares during treatment (%)	20 (63)	14 (40)	0.067
Time of flare †	36 (4-78)	60 (4-78)	0.27
peak value flare* (x ULN)	13.7±6.9	16.4±13.8	0.89
Response (%)	10 (31)	10 (29)	0.81

Table 1 and table 2: *Mean (SD). †Median (range).

Peg, pegylated; IFN, interferon; ALT, alanine aminotransferase; ULN, upper limit of normal; HBV, hepatitis B virus.

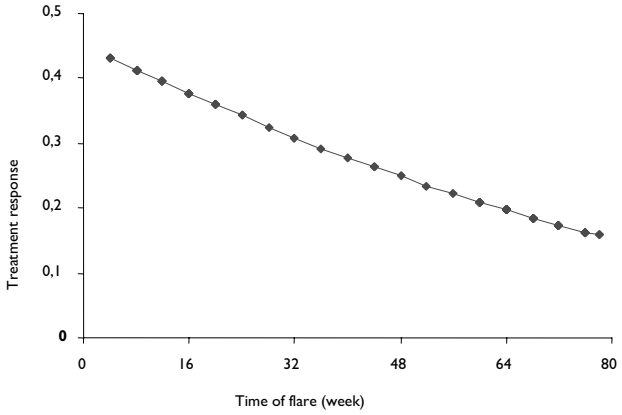

Figure 1. Proportion of response in relation to the time of the flare. Early presence of a flare increased the chance of response (p = 0.081). Probability of response is shown on the y axis.

Flares in relation to response to treatment and genotype

Among the 67 flare patients, 20 (30%) responded to therapy, 10 (31%) in the monotherapy and 10 (29%) in the combination group. Eight patients (12%) exhibited loss of HBsAg at the end of follow up. On-treatment flares led more often to treatment response (41%) than post-treatment flares (21%) (p = 0.081, Figure 1).

The frequency of flares was comparable for patients with different HBV genotypes (Figure 2A). However, the timing of flares and response to therapy differed among the HBV genotypes. A total of 17 (74%) flares were recorded ontreatment in patients harbouring genotype A, versus three (43%) in genotype B, four (33%) in genotype C, and eight (27%) in genotype D (genotype A v other genotypes, p = 0.046) (Figure 2B). Treatment response in the flare population was 47% for genotype A, 28% for B, 18% for C, and 19% for D (genotype A v genotype D, p = 0.05) (Figure 2C). In addition to the timing of the flares and HBV genotype, the magnitude of the ALT elevation was associated with treatment response. Mean ALT during the flare within responders was 20.1 xULN versus 13.2 xULN in non-responders (p = 0.036).

Host induced versus virus induced flares

Close online monitoring of serum ALT and HBV DNA levels revealed different patterns of exacerbation (Figure 3). A flare was defined as "virus induced" when preceded by an increase of least 1 log HBV DNA within four months. In general, these flares did not lead to a decline in serum HBV DNA. A flare was defined as "host induced" when the preceding HBV DNA levels were stable and when the flare was followed by a decline of 1 log HBV DNA or more within the four months thereafter. Flares that did not meet one of these criteria were classified as indeterminate. Only the first occurring flares were classified.

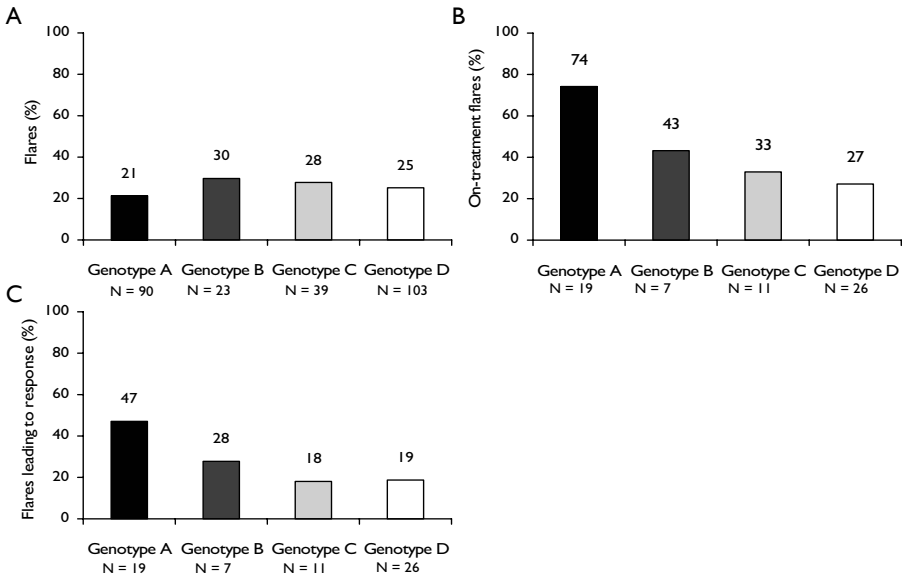

Figure 2. (A) Frequency of flares according to hepatitis B virus (HBV) genotype. Among the most important genotypes in our study (HBV genotypes A, B, C and D (n = 255)), no significant difference in the frequency of flares was found. (B) Proportion of flares recorded during treatment among the flare population according to HBV genotype (n= 63). On-treatment flares predominantly occurred within genotype A. Genotype A versus genotype D, p = 0.029. (C) Flares leading to response according to genotype (n=63). Genotype A versus genotype D, p = 0.050.

For both host and virus induced flares, a minimum of 1 log HBV DNA alternation was chosen to exclude random oscillation of serum HBV DNA as a basis for our flare criteria. Twenty four flares (36%) were characterised as host induced, 25 (38%) were virus induced, and 17 (26%) were indeterminate. One flare could not be classified due to missing HBV DNA levels. Among the 67 flare patients, a host induced flare was strongly related to response to therapy (Figure 4). Fourteen of the 24 patients (58%) with a host induced flare responded to therapy compared with five of 25 patients (20%) with a virus induced and one patient (6%) with an indeterminate flare. Moreover, eight patients (33%) with a host induced flare, but none of those with a virus induced or indeterminate flare, were HBsAg negative at the end of follow up. Seventy five percent of host induced flares versus 16% of virus induced flares occurred during treatment ($p < 0.0001$). Median peak of ALT of host induced flares was 13.8 xULN (range 5.3–60) and 12.1 xULN (range 3–45) for virus induced flares. Eleven patients (61%) with a host induced flare during treatment and three (50%) with a host induced flare after treatment responded to therapy. One patient (25%) with a virus induced flare during treatment and four (19%) with a virus induced flare after treatment responded to therapy. Within the flare population, multivariate analysis showed that the occurrence of host induced flare ($p = 0.043$; relative risk 3.5; 95% CI 0.9–13.9) and the

magnitude of the ALT elevation (p = 0.031; RR 1.1; 95% CI 1.0–1.1) were the only factors independently predictive of response (serum HBeAg loss). On-treatment flares were not significantly related to response in this analysis (p = 0.65; RR 1.4; 95% CI 0.3–6.7).
After entering the occurrence of host induced flares in the previously described multivariate analysis[8] of the total study population (n=266), it remained a significant variable predicting response (relative risk 2.4; 95% CI 1.0–5.8; p = 0.05).

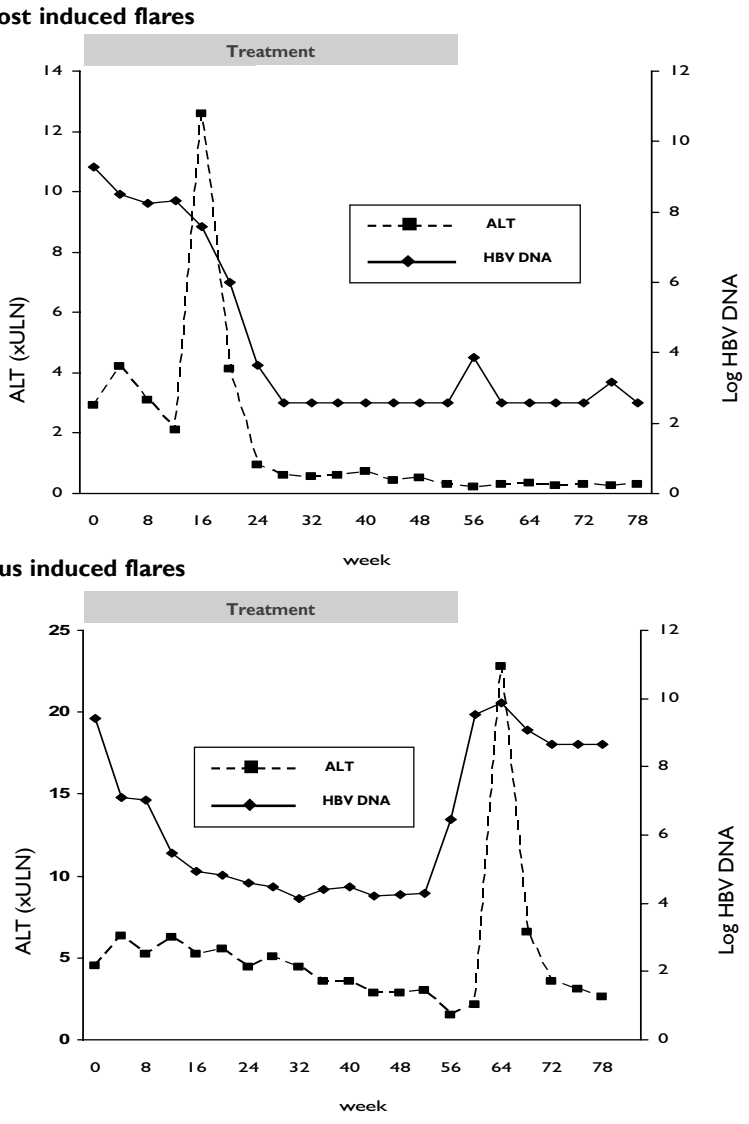

Figure 3. (A) Case with host induced flare. The elevation in serum alanine aminotransferase (ALT) was followed by a decrease in viral load (log hepatitis B virus (HBV) DNA). (B) Case with virus induced flare. Serum ALT elevation was preceded by a sharp increase in serum HBV DNA.

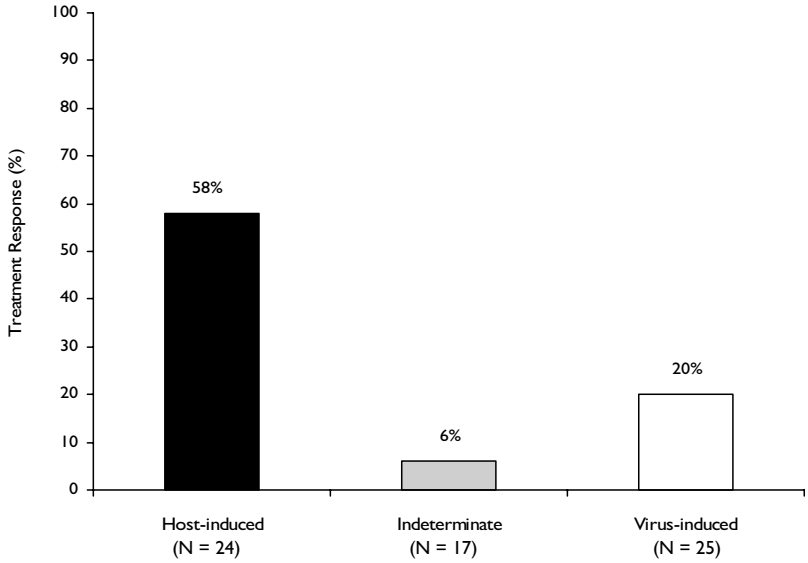

Figure 4. Host induced, indeterminate, and virus induced flares in relation to treatment response. Host induced versus virus induced, p = 0.008.

DISCUSSION

Spontaneous or treatment induced flares of inflammation are frequently observed in CHB. These abrupt elevations in serum ALT are the result of an increase in intrahepatic necroinflammation associated with expanded numbers of intrahepatic lymphocytes, in particular cytotoxic T lymphocytes. Cytotoxic T lymphocytes are important to control HBV but can also induce liver damage, depending on the environment and functional capability[10–15]. Therapy with IFN is based on its stimulating effect on cytotoxic T lymphocyte and natural killer cell function. Flares during standard IFN treatment occur typically during the second and third month, and are thought to herald virological response and disease remission[2–5 16]. Probably, these flares represent an attempt of the immune system to clear the HBV infection.

In the current study, 29% of patients experienced a flare during therapy (n=34) or follow up (n=33). We did not find a significant difference between the number of flares in patients treated with Peg-IFN α-2b alone versus those treated with Peg-IFN α-2b in combination with lamivudine. Patients with low baseline ALT or pre-existing cirrhosis were more prone to having flares. Cirrhotics tended to experience flares with high ALT values. These patients should be monitored carefully during treatment with Peg-IFN α-2b, not only because of their increased risk of flares but also because of their diminished residual liver function and

the consequent risk of developing decompensated liver disease. In the current study, no permanent or life threatening signs of liver failure were encountered.

Overall, flares were not associated with response to therapy. However, flares during treatment were more often associated with response than flares after treatment (Figure 1). In addition to the timing of flares, response was dependent on HBV genotype and the magnitude of the flare. Previously, a strong association between the severity of flares and HBsAg seroconversion was found both in the natural history of CHB and in the setting of IFN therapy[16 17]. In these studies, different definitions of flares were used. Nair and Perrillo[16] defined a flare as an increase in ALT of at least twice the ULN compared with baseline values while Yuen and colleagues[17] defined a flare as elevated transaminases above twice the ULN. As our patients already had high baseline serum ALT levels (ALT levels above twice the ULN was used as an entry criterion in this study population), these definitions were less suitable. For a clear distinction between flares and relative mild elevations in serum ALT, we based our definition on our previous experience, in which a threefold increase in serum ALT from baseline was used[7].

An important finding of the current study was the distinct patterns of flares occurring, with stable viral load followed by a decrease in viral load (host induced flares) versus flares preceded by an increase in viral load and variable viral loads afterwards (virus induced flares). Patients with host induced flares responded significantly better to therapy than those with a virus induced flare. Multivariate analysis revealed host induced flare as the only independent factor predicting treatment response. Interestingly, all patients undergoing HBsAg seroconversion had a host induced flare. This further supports the hypothesis that full control and elimination of the virus, as indicated by clearance of HBeAg and HBsAg, is achieved by a vigorous host immune response rather than by direct suppression of the virus. Previous studies have shown that both spontaneous or IFN-α induced exacerbations of hepatocellular necrosis in CHB are associated with induction of a virus specific CD4+ T cell response[18 19]. Under IFN-α therapy such a hepatitis flare preceding sustained HBeAg seroconversion requires a substantial increase in IL-12 production, along with induction of the Th1 cytokines IFN-α and IL-2[20]. Virus induced flares, which emerged after increasing levels of HBV DNA, were related to treatment with the combination with lamivudine, and more frequently seen after therapy. These flares were attributed to reactivation of HBV after withdrawal of lamivudine. In general, they did not lead to disease remission but have been associated with clinical exacerbation and disease progression[7]. In our study, virus induced flares did not usually lead to response, and even appeared to be detrimental rather than beneficial for treatment response.

Virus induced flares are not restricted to CHB patients treated with IFN and or lamivudine, but also occur during the natural history of the disease. Liu et al described several patients in whom significant flares were preceded by an increase in HBV DNA[21].

Studies in anti-HBe positive patients also showed episodes of flares as a result of sudden reactivation of HBV[22–24.]

In general, flares during treatment with IFN or Peg-IFN α-2b should not be treated with nucleoside analogues, and IFN should only be discontinued in case of impending liver failure. Particular care should be taken in patients with cirrhosis who have the highest risk of developing liver failure. On-treatment flares are likely to be host or IFN induced flares, and could well herald loss of HBeAg or even HBsAg. In contrast, flares after treatment, especially after lamivudine, are in general detrimental flares. These flares are typically seen after an increase in HBV DNA and seldom lead to treatment response. Retreatment with a nucleoside analogue should then be considered.

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REFERENCES

1. Lee W. Hepatitis B virus infection. N Eng J Med 1997;337:1733–45.
2. Peters M, Davis GL, Dooley JS, et al. The interferon system in acute and chronic viral hepatitis. Prog Liver Dis 1986;8:453–67.
3. Perrillo RP. Acute flares in chronic hepatitis B: the natural and unnatural historyof an immunologically mediated liver disease. Gastroenterology 2001;120:1009–22.
4. Perrillo RP, Schiff ER, Davis GL, et al. A randomized, controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of chronic hepatitis B. The Hepatitis Interventional Therapy Group. N Engl J Med 1990;323:295–301.
5. Alexander GJ, Brahm J, Fagan EA, et al. Loss of HBsAg with interferon therapy in chronic hepatitis B virus infection. Lancet 1987;2:66–9.
6. Tang TJ, Kwekkeboom J, Mancham S, et al. Combined cytolytic and noncytolytic intrahepatic CD8 T-lymphocyte reactivity is important for response to antiviral therapy in chronic hepatitis B patients. Hepatology 2003;38(suppl 1):254A.
7. Honkoop P, de Man RA, Niesters HG, et al. Acute exacerbation of chronic hepatitis B virus infection after withdrawal of lamivudine therapy. Hepatology 2000;32:635–9.
8. Janssen H, Van Zonneveld M, Senturk H, et al. Pegylated interferon alpha-2b alone or in combination with lamivudine as the treatment for HBeAg-positive chronic hepatitis B. Lancet 2005;365:123–9.
9. Pas SD, Fries E, De Man RA, et al. Development of a quantitative real-time detection assay for hepatitis B virus DNA and comparison with two commercial assays. J Clin Microbiol 2000;38:2897–901.
10. Lowin B, Hahne M, Mattmann C, et al. Cytolytic T-cell cytotoxicity is mediated through perforin and Fas lytic pathways. Nature 1994;370:650–2.
11. Milich DR, McLachlan A, Stahl S, et al. Comparative immunogenicity of hepatitis B virus core and E antigens. J Immunol 1988;141:3617–24.
12. Yang PM, Su IJ, Lai MY, et al. Immunohistochemical studies on intrahepatic lympho cyte infiltrates in chronic type B hepatitis, with special emphasis on the activation status of the lymphocytes. Am J Gastroenterol 1988;83:948–53.
13. Tsai SL, Chen PJ, Lai MY, et al. Acute exacerbations of chronic type B hepatitis are accompanied by increased T cell responses to hepatitis B core and e antigens. Implications for hepatitis B e antigen seroconversion. J Clin Invest 1992;89:87–96.
14. Bertoletti A, Maini MK. Protection or damage: a dual role for the virus-specific cytotoxic T lymphocyte response in hepatitis B and C infection? Curr Opin Microbiol 2000;3:387–92.
15. Bertoletti A, Maini M, Williams R. Role of hepatitis B virus specific cytotoxic T cells in liver damage and viral control. Antiviral Res 2003;60:61–6.

16. Nair S, Perrillo RP. Serum alanine aminotransferase flares during interferon treatment of chronic hepatitis B: is sustained clearance of HBV DNA dependent on levels of pretreatment viremia? Hepatology 2001;34:1021–6.
17. Yuen MF, Yuan HJ, Hui CK, et al. A large population study of spontaneous HBeAg seroconversion and acute exacerbation of chronic hepatitis B infection: implications for antiviral therapy. Gut 2003;52:416–19.
18. Lohr HF, Weber W, Schlaak J, et al. Proliferative response of CD4+ T cells and hepatitis B virus clearance in chronic hepatitis with or without hepatitis B eminus hepatitis B virus mutants. Hepatology 1995;22:61–8.
19. Marinos G, Torre F, Chokshi S, et al. Induction of T-helper cell response to hepatitis B core antigen in chronic hepatitis B: a major factor in activation of the host immune response to the hepatitis B virus. Hepatology 1995;22:1040–9.
20. Rossol S, Marinos G, Carucci P, et al. Interleukin-12 induction of Th1 cytokines is important for viral clearance in chronic hepatitis B. J Clin Invest 1997;99:3025–33.
21. Liu CJ, Chen PJ, Lai MY, et al. A prospective study characterizing full-length hepatitis B virus genomes during acute exacerbation. Gastroenterology 2003;124:80–90.
22. Perrillo RP, Campbell CR, Sanders GE, et al. Spontaneous clearance and reactivation of hepatitis B virus infection among male homosexuals with chronic type B hepatitis. Ann Intern Med 1984;100:43–6.
23. Mels GC, Bellati G, Leandro G, et al. Fluctuations in viremia, aminotransferases and IgM antibody to hepatitis B core antigen in chronic hepatitis B patients with disease exacerbations. Liver 1994;14:175–81.
24. Davis GL, Hoofnagle JH, Waggoner JG. Spontaneous reactivation of chronic hepatitis B virus infection. Gastroenterology 1984;86:230–5.

Chapter 4

Treatment with peginterferon α-2b for HBeAg-positive chronic hepatitis B: HBsAg loss Is associated with HBV genotype

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ABSTRACT

Background and aims: Hepatitis B surface antigen (HBsAg) loss is the hallmark of a complete response to antiviral therapy for chronic hepatitis B. In this study, we investigated the frequency of HBsAg loss after treatment with peginterferon α-2b.

Methods: In a multicenter randomized controlled trial, 266 HBeAg-positive patients were treated for 52 wks with Peg-IFN α-2b (100 µg/wk) in combination with either lamivudine (100 mg/day) or placebo. Post-treatment follow-up was 26 wks.

Results: At the end of follow-up, 95 (36%) of the 266 patients exhibited HBeAg loss, 18 (7%) HBsAg loss, and 16 (6%) HBsAg seroconversion. Addition of lamivudine did not enhance HBeAg loss, HBsAg loss, or development of anti-HBs. All 18 patients who showed HBsAg loss had normal ALT; 11 (61%) of these patients were also hepatitis B virus (HBV) DNA negative (< 400 copies/mL) at the end of follow-up. Loss of HBsAg differed according to HBV genotype: 14% for genotype A, 9% for genotype B, 3% for genotype C, and 2% for genotype D (A vs D: $p = 0.006$).

Conclusions: One year of Peg-IFN α-2b for HBeAg-positive patients led to HBsAg loss in 7%. Our study indicates that treatment with Peg-IFN α-2b is the best therapy to achieve HBsAg clearance in patients with genotype A.

INTRODUCTION

Worldwide, over 360 million people are chronically infected with hepatitis B virus (HBV)[1]. Chronic HBV infection, defined as hepatitis B surface antigen (HBsAg) positivity for more than 6 months, is associated with increased risk of developing cirrhosis, hepatic decompensation, and hepatocellular carcinoma[2,3]. Loss of HBsAg is closely related to improved long-term histological and virological outcome as well as decreased risk of hepatic decompensation and hepatocellular carcinoma[4–7]. Therefore, the ultimate purpose of therapy for chronic hepatitis B is serum HBsAg negativity.

Treatment with nucleoside or nucleotide analogues is associated with reactivation of disease activity after termination of treatment or, in case of continued therapy, development of HBV variants which are resistant to treatment. Several studies with lamivudine have shown absence or less than 2% loss of HBsAg [8–11]. One-year treatment with adefovir dipivoxil resulted in 1.6% loss of HBsAg, a percentage in the same range as spontaneous HBsAg seroclearance[12,13]. Treatment with standard interferon α may lead to increased loss of HBsAg [14,15]. To date, little is known about loss of HBsAg due to treatment with pegylated (peg)interferon α. Therefore, the aim of the studywas to investigate the incidence of and the factors predicting HBsAg loss due to treatment with peginterferon α-2b alone or in combination with lamivudine.

PATIENTS AND METHODS

This study was conducted as an ancillary study of a multicenter trial comparing combination therapy of peginterferon α-2b and lamivudine versus peginterferon α-2b alone[16]. For this study, 266 patients were analyzed after central evaluation of eligibility. Males and females were included if they were 16 yr or older, had been positive for HBsAg and HBeAg for at least 6 months prior to randomization, and had elevated serum ALT levels of at least twice the upper limits of normal (ULN) 2 months before randomization. Exclusion criteria included antiviral or immune modulatory treatment within 6 months; antibodies against hepatitis C, hepatitis D, or human immunodeficiency virus; presence of decompensated liver disease (prothrombin time prolonged by >3 s, serum albumin <35 g/L, ascites, encephalopathy, history of variceal bleeding); pregnancy or recent drug or alcohol abuse. Other exclusion criteria were: inadequate hematological levels, i.e., leucopenia ($\leq$ 3,000/mm^3), thrombocytopenia ($\leq$ 100,000/mm^3), or granulocytopenia ($\leq$ 1,800/mm^3); serum α-fetoprotein level of more than 50 ng/mL; hypo- or hyperthyroidism; radiological evidence of hepatocellular carcinoma, or any contraindication specified for interferon. All patients gave written informed consent and the ethics committee at the participating centers approved the protocol.

Study Design

The patients received 100 µg/wk peginterferon α-2b for the first 32 wks, followed by 50 µg/wk peginterferon α-2b from week 32 to week 52. This was combined with either 100 mg lamivudine daily or a placebo. Post-treatment follow-up lasted 26 wks.

During therapy and follow-up, patients were seen monthly for routine examination as well as biochemical and HBV DNA assessment. Transaminases were determined locally and are therefore expressed as times the ULN. HBV DNA was assessed using in-house Taqman PCR (detection limit 400 copies/mL) based on the Eurohep standard[17]. HBeAg (AxSYM, Abbott, Abbot Park, IL, USA) and HBsAg (AxSYM, Abbott) status were measured at weeks 0, 32, 52 (during treatment) and week 78 (end of follow-up). HBV genotype (Inno-Lipa Assay, Innogenetics) was assessed at baseline. HBeAg and HBsAg responses were defined as loss of serum HBeAg and HBsAg at the end of follow-up, respectively. HBs- and HBe seroconversion were defined as loss of HBs or HBe antigen, respectively, with development of the corresponding antibodies. Liver histology was assessed at baseline and an optional biopsy was performed at the end of treatment. Paired biopsies were available for 110 patients. Histological scoring was performed by one experienced pathologist according to the histological activity index, modified by Ishak et al.[18]. The pathologist was blinded for chronological order of biopsies and treatment schedule. Improvement of histology was defined as a reduction of at least two points in the necroinflammatory score (range 0–18) and one point in the fibrosis score (range 0–6).

Statistical Analysis

All datawere analyzed by means of SPSS version 10.1 (SPSS Inc., Chicago, IL). Chi-square or Fisher's exact test and the Mann-Whitney U test were used where appropriate (all 2-tailed). A p value < 0.05 was considered significant.

RESULTS

Loss of HBeAg and HBsAg

At the end of follow-up, 95 (36%) of the 266 patients exhibited HBeAg loss, 18 (7%) loss of HBsAg, and 16 (6%) HBsAg seroconversion. Among patients with HBeAg loss, 19% were also HBsAg negative at the end of follow-up. Addition of lamivudine did not enhance HBeAg loss, HBsAg loss, or development of anti-HBs. At the end of follow-up, HBeAg clearance was found for 36% in the monotherapy and 35% of the combination group. HBsAg clearance at the end of follow-up was 7% for both treatment groups. Results of HBeAg and HBsAg testing were available for weeks 0, 32, 52, and 78. Percentages of HBeAg- and HBsAg clearance and HBV DNA < 400 copies/mL at these time points are shown in Figure 1.

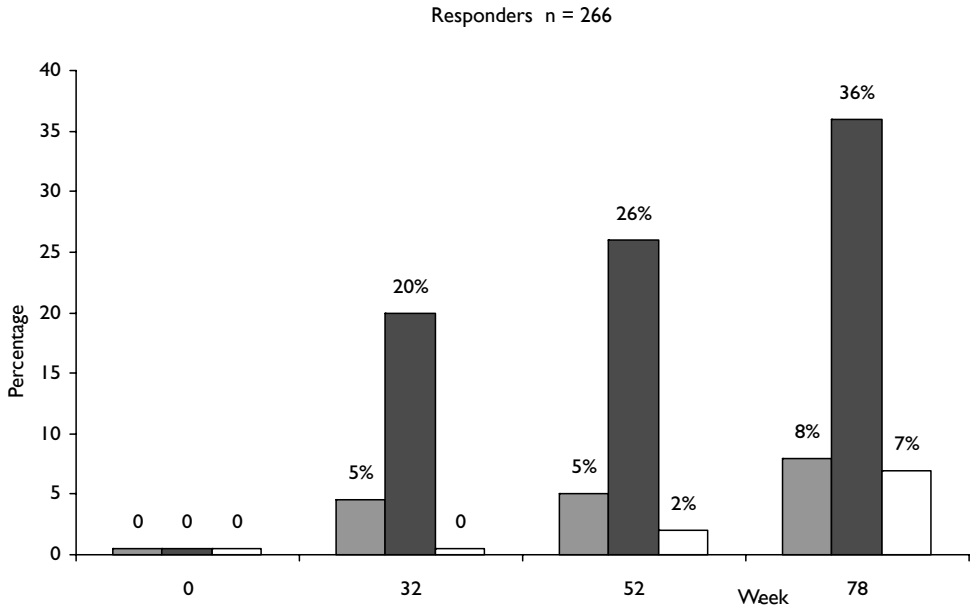

Figure 1. Course of serum HBeAg, HBsAg, and PCR-negativity (< 400 copies/mL) during treatment and follow-up. Data for both Peginterferon/placebo and Peginterferon/lamivudine were combined. Percentages were calculated for patients who were HBeAg or HBsAg responders at the end of follow-up.

Twelve patients became HBsAg negative before week 52 (end of treatment) and 6 became HBsAg negative between week 52 and week 78 (end of follow-up). Baseline characteristics for nonresponders and for those who showed loss of serum HBeAg and/or HBsAg are given in Table 1. Compared to nonresponders, baseline HBV DNA values were lower among those exhibiting HBeAg loss ($p = 0.043$), but not those with HBsAg loss. Compared to nonresponders and HBeAg responders, patients with HBsAg loss were significantly older ($p = 0.001$).

Loss of HBeAg and HBsAg in Relation to HBV DNA, ALT, and Histology

Among HBeAg responders, 59 (65%) patients had normal serum ALT, 62 (69%) HBV DNA <200,000 copies/mL, and 9 (22%) HBV DNA < 400 copies/mL by PCR at the end of follow-up (Figure 2). No differences were found between the different treatment regimens and HBV DNA response or ALT normalization. Among HBsAg responders, all patients had normal serum ALT and HBV DNA < 200,000 copies/mL, and 11 (61%) had HBV DNA <400 copies/mL by PCR at the end of follow-up. In the nonresponder group, we found 28 (18%) patients with normal serum ALT, 12 (9%) withHBV DNA < 200,000 copies/mL, and none with HBV DNA < 400 copies/mL by PCR at the end of follow-up.

Table 1. Patient data on non-responders, HBeAg responders, and HBsAg responders

Characteristics	Non-responders (n = 171)	HBeAg neg. (n = 95)	P =	HBsAg Pos. (n = 248)	HBsAg neg. (n = 18)	P =
Age (yrs)†	34 ± 12.9	38 ± 12.9	0.07	34 ± 12.6	45 ± 12.8	0.001
Male (%)	136 (80)	69 (73)	0.20	190 (77)	15 (83)	0.5
Weight (kgs) †	73 ± 12.9	73 ± 16.5	0.98	73 ± 14.2	76 ± 14.4	0.3
ALT (x ULN) †	4.1 ± 3.5	4.7 ± 3.2	0.18	4.3 ± 3.5	3.8 ± 1.6	0.2
Log HBV DNA†	9.2 ± 1.0	8.9 ± 0.9	0.04	9.1 ± 1.0	9.2 ± 0.7	0.4
Histology *						
Fibrosis	2 (0-6)	3 (0-6)	0.01	3 (0-6)	3 (0-6)	0.05
Necroinflammation	5 (1-10)	6 (2-10)	<0.001	5 (1-10)	7 (4-9)	0.02
Race (%)						
Caucasian	127 (74)	69 (73)	0.39	182 (73)	14 (78)	0.98
Asian/ Mongoloid	35 (21)	17 (18)		49 (20)	3 (17)	
Other	9 (5)	9 (9)		17 (7)	1 (6)	
Area of enrollment (%)						
North. & West. Europe	62 (36)	44 (46)	0.12	96 (39)	10 (56)	0.34
Eastern Europe	17 (10)	13 (14)		26 (11)	4 (22)	
Mediterranean	59 (35)	24 (25)		81 (33)	2 (11)	
East Asia	23 (14)	6 (6)		27 (11)	2 (11)	
North America	10 (6)	8 (8)		18 (7)	0	

†Mean ± SD. *Median (range).

As far as histology is concerned, improved (reduction of >2 points HAI) necroinflammation scores were found for 36 (38%) of the HBeAg responders, 7 (39%) of the HBsAg responders, and 39 (23%) of the nonresponders (HBeAg and HBsAg responders versus nonresponders, p = 0.016). Among HBsAg-negative patients, the fibrosis score had improved in 2 cases and worsened in 3 cases, while 3 patients showed no change (median 0, range -2–1). Fibrosis scores were not significantly different among nonresponders, HBeAg responders, and HBsAg responders.

Loss of HBeAg and HBsAg in relation to genotype

The most common HBV genotypes were genotype A (n=90), B (n=23), C (n=39), and D (n =103) (Table 2). HBV genotypes A (97%) and D (95%) were found predominantly among Caucasians; among Asians, genotypes B (78%) and C(80%)were the most common (Table 2). Patients with genotype A lived in Northwest and Eastern Europe, patients with genotype B or C in all continents, and those with genotype D primarily in the Mediterranean countries and Northwest Europe.

HBV genotype was not only associated with HBeAg response, but also with HBsAg response (Figure 3). Loss of HBsAg was found predominantly in patients harboring genotype A. Thirteen of the 90 genotype A patients (14%), 2 of the 23 genotype B (9%), 1 of the 39 genotype C (3%), and 2 of the 103 genotype D (2%) patients had lost HBsAg at the end of follow-up (genotype A versus genotype D, p = 0.006). Anti-HBs was seen in 12 patients with genotype A (13%), 2 with genotype B (9%), 0 with genotype C, and 2 with genotype D (2%). Among HBeAg responders, 31% of genotype A, 20% of genotype B, 9% of genotype C, and 8% of genotype D patients lost serum HBsAg as well; anti-HBs was seen in 29%, 20%, 0%, and 8% at the end of follow-up for genotypes A, B, C, and D, respectively.

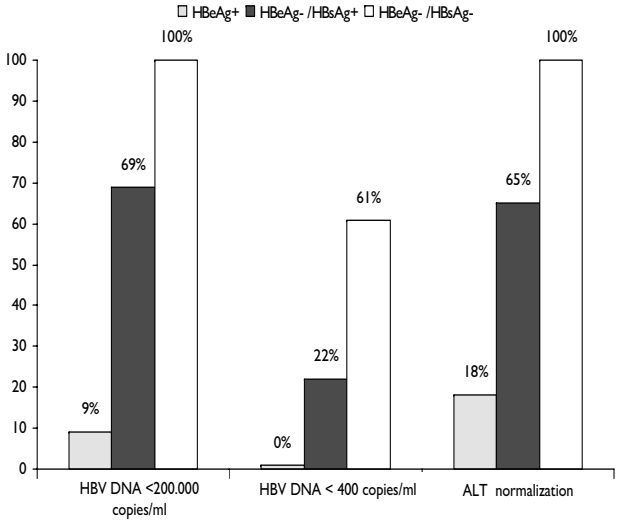

Figure 2. HBV DNA response and ALT normalization at the end of follow-up in HBeAg-positive (n= 171), HBeAg-negative (n=95), and HBsAg-negative (n=18) patients at the end of follow-up. HBeAg-positive versus HBeAg-negative patients: for HBV DNA <200,000 copies/mL, p < 0.0001; HBV DNA <400 copies/mL, p = ns; and ALT normalization, p < 0.0001. HBeAg-negative versus HBsAg-negative: for HBV DNA <200,000 copies/mL, p = 0.034; HBV DNA <400 copies/mL, p < 0.0001; and ALT normalization p = 0.002.

Table 2. Baseline characteristics and geographical distribution of patients with HBV Genotype A, B, C, or D

Characteristics	HBV Genotype A N = 90	B N = 23	C N = 39	D N = 103
Age (yrs)†	43 ± 14.1	33 ± 8.1	35 ± 10.0	29 ± 10.0
Weight (kgs) †	77 ± 12.8	64 ± 10.1	68 ± 11.8	74 ± 15.2
ALT (x ULN) †	4.2 ± 2.6	4.2 ± 2.2	3.9 ± 2.8	4.6 ± 4.5
Log HBV DNA†	9.1 ± 0.8	8.3 ± 1.4	8.3 ± 0.9	9.5 ± 0.9
Histology*				
Fibrosis	3 (0-6)	3 (0-6)	3 (0-6)	2 (0-6)
Necroinflammation	6 (2-10)	6 (3-8)	5 (2-10)	4 (1-10)
Race (%)				
Caucasian	87 (97)	1 (4)	2 (5)	98 (95)
Asian/Mongoloid	1 (1)	18 (78)	31 (80)	0
Other	2 (2)	4 (17)	6 (15)	5 (5)
Area of enrollment (%)				
North. & West. Europe	55 (61)	7 (30)	12 (31)	24 (23)
Eastern Europe	28 (31)	0	0	2 (2)
Mediterranean	5 (6)	0	0	77 (75)
East Asia	0	11 (48)	17 (44)	0
North America	2 (2)	5 (22)	10 (26)	0

†Mean ± SD. *Median (range).

DISCUSSION

The ultimate endpoint of antiviral therapy for chronic HBV infection is loss of HBsAg, which is accompanied not only by disease remission in terms of ALT normalization, but also by a significantly decreased risk of liver failure and hepatocellular carcinoma[4–6]. Spontaneous HBsAg loss is uncommon and varies from 1% to 2% annually[19].

In the current study, after 52 wks of peginterferon α-2b therapy in combination with lamivudine or placebo and 26 wks of follow-up, we found serum HBsAg loss in 18 patients (7%) and development of anti-HBs in 16 patients (6%). Adding lamivudine did not enhance HBeAg- or HBsAg seroclearance. Previous studies have shown that treatment with nucleoside or nucleotide analogues, such as lamivudine and adefovir, probably do not lead to an enhanced

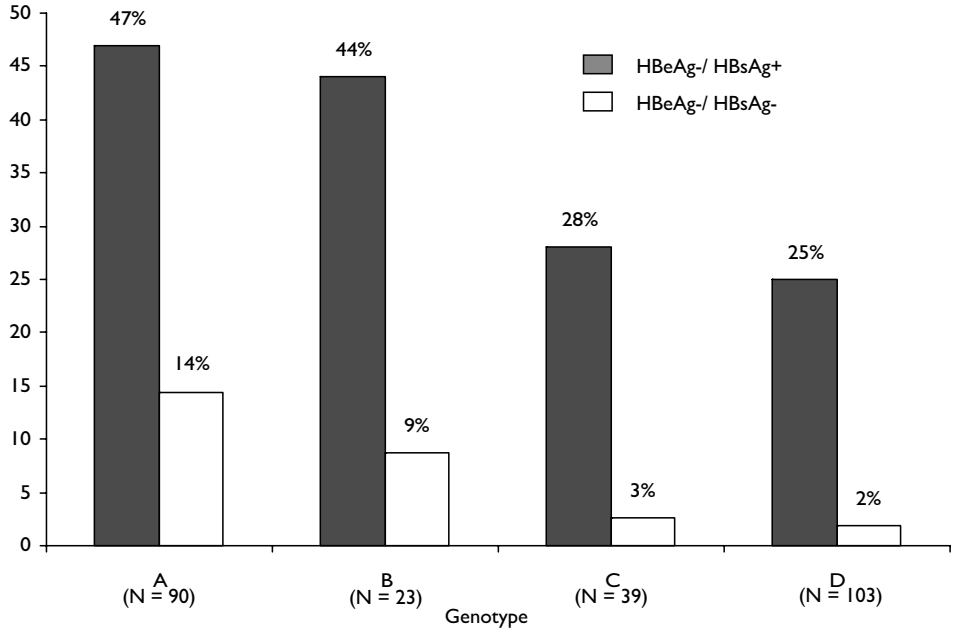

Figure 3. Proportion of HBeAg- and HBsAg clearance at the end of follow-up according to genotype (n = 266). *Genotype A versus D, p = 0.006.

rate of HBsAg seroclearance [8–11,20,21]. Nevertheless, in several single cases, prolonged adefovir therapy induced a sharp reduction of cccDNA and HBsAg seroconversion [22]. Therefore, the hypothesis that a decrease in intrahepatic viral load itself may be associated with restoration of a noncytolytic Th1 response and HBsAg seroconversion deserves further investigation[23]. A recent pilot study using tenofovir did show HBsAg seroconversion in 5 of 35 (14%) patients[24]. This needs to be confirmed in a large randomized study which was initiated recently.

We previously demonstrated that HBeAg seroconversion after treatment with standard interferon α persisted longer than after lamivudine therapy[25]. Furthermore, HBsAg seroconversion occurred more frequently in patients treated with interferon α than those receiving lamivudine. In keeping with the sustained response after interferon α therapy, a prolonged follow-up study by van Zonneveld showed that over a median period of 8.8 yr, the HBsAg seroconversion rate increased up to 52% in chronic hepatitis B patients who responded to interferon therapy[26]. Overall, these results support the hypothesis that peg-interferon α, more than nucleoside analogs, can induce a complete and vigorous immune response which leads not only to loss of HBeAg, but also to loss of HBsAg.

Detailed analysis of our data showed that both HBeAg and HBsAg seroconversion were strongly associated with the HBV genotype. Among responders harboring genotype A,

there was an HBsAg seroclearance rate of 31%. Genotype A patients exhibited a high rate of HBeAg and HBsAg loss, not only as a result of peginterferon α therapy but also spontaneously[27]. This probably relates to the presence of genotype-dependent mutations llocated within the basic core promotor region of the genome[28]. Among Asian patients, we found a higher HBeAg- and HBsAg seroconversion rate for those with genotype B than for those with genotype C. This confirms the results of previous studies that showed better results of interferon α for patients with genotype B, also due to a response predisposition caused by core promotor mutations[29–32]. Although our results revealed a strong association between HBV genotype and HBsAg loss, the absolute number with HBsAg clearance is relatively low. Therefore, multivariate analysis for the prediction of HBsAg loss could not be performed. Nevertheless, the clear association between HBV genotype and loss of HBsAg should be taken into account in future studies.

Two global chronic hepatitis B studies on peginterferon α-2a, in a similar therapeutic regimen as our study, have recently been finalized. In a difficult-to-treat HBeAg-negative population, Marcellin et al. found4% HBsAg seroconversion[33]. This low HBsAg response can probably be explained by the fact that HBeAg-negative patients often harbor the non-A genotype and carry pre-core mutations which may hamper effective immune stimulation by interferon α. In an HBeAg-positive population treated for 1 yr with peginterferon α-2a, Lau and colleagues found 32% HBeAg seroconversion, 3% HBsAg loss, and 3% HBsAg seroconversion[21]. When this rate of HBsAg loss is compared with our results, it is important to note that 87% of their population were Asian, for whom low HBsAg seroclearance rates after interferon á have been described previously[21]. The HBsAg loss among our Asian patients was 5%. An interesting finding of our study is the relatively large number of patients (39%) for whom serum HBV DNA could still be detected by Taqman technology after HBsAg seroclearance. Yuen et al. recently described a Chinese chronic HBV population with HBsAg seroclearance, spontaneously, or after interferon α therapy. Upon long-term follow-up, they found serum HBV DNA in only 2%, but intrahepatic HBV DNA (mainly cccDNA) in 37% of the population[4]. This study and earlier studies showing a delay between postinterferon α HBsAg seroclearance and HBV DNA negativity by PCR[4, 34] suggest that with continued follow-up, HBV DNA might further disappear from serum in most patients who become HBsAg negative. This is further supported by analysis of our data from a previous study, which showed that 90% of the HBsAg-negative patients also had undetectable HBV DNA levels by PCR after prolonged follow-up[26].

In conclusion, 7% of patients treated with peginterferon α-2b showed HBsAg loss, a response higher than that reported for lamivudine and/or adefovir. HBV genotypes were associated with HbeAg and HBsAg seroclearance. In particular, patients with genotype A seemed to benefit from peginterferon α-2b therapy, with an HBsAg clearance rate of 14% overall and 31% among responders who had lost HBeAg.

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REFERENCES

1. Lai CL, Ratziu V, Yuen MF, et al. Viral hepatitis B. Lancet 2003;362(9401):2089–94.
2. Ganem D, Prince AM. Hepatitis B virus infection—Natural history and clinical consequences. N Engl J Med 2004;350(11):1118–29.
3. Lee WM. Hepatitis B virus infection. N Engl J Med 1997;337(24):1733–45.
4. Yuen MF, Wong DK, Sablon E, et al. HBsAg seroclearance in chronic hepatitis B in the Chinese: Virological, histological, and clinical aspects. Hepatology 2004;39(6):1694–701.
5. Yang HI, Lu SN, Liaw YF, et al. Hepatitis B e antigen and the risk of hepatocellular carcinoma. N Engl J Med 2002;347(3):168–74.
6. Beasley RP. Hepatitis B virus. The major etiology of hepatocellular carcinoma. Cancer 1988;61(10):1942–56.
7. Chen YC, Sheen IS, Chu CM, et al. Prognosis following spontaneous HBsAg seroclearance in chronic hepatitis B patients with or without concurrent infection. Gastroenterology 2002;123(4):1084–9.
8. Lai CL, Chien RN, Leung NW, et al. A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. N Engl J Med 1998;339(2):618.
9. Dienstag JL, Schiff ER, Wright TL, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med 1999;341(17):1256–63.
10. Lau DT, Khokhar MF, Doo E, et al. Long-term therapy of chronic hepatitis B with lamivudine. Hepatology 2000;32(4Pt 1):828–34.
11. Perrillo RP, Lai CL, Liaw YF, et al. Predictors of HBeAg loss after lamivudine treatment for chronic hepatitis B. Hepatology 2002;36(1):186–94.
12. Kato Y, Nakao K, Hamasaki K, et al. Spontaneous loss of hepatitis B surface antigen in chronic carriers, based on a long-term follow-up study in Goto Islands, Japan. J Gastroenterol 2000;35(3):201–5.
13. Fattovich G, Giustina G, Sanchez-Tapias J, et al. Delayed clearance of serum HBsAg in compensated cirrhosis B: Relation to interferon alpha therapy and disease prognosis. European Concerted Action on Viral Hepatitis (EUROHEP). Am J Gastroenterol 1998;93(6):896–900.
14. Janssen HL, Gerken G, Carreno V, et al. Interferon alfa for chronic hepatitis B infection: Increased efficacy of prolonged treatment. The European Concerted Action on Viral Hepatitis (EUROHEP). Hepatology 1999;30(1):238–43.
15. Korenman J, Baker B, Waggoner J, et al. Long-term remission of chronic hepatitis B after alpha-interferon therapy. Ann Intern Med 1991;114(8):629–34.
16. Janssen HL, van Zonneveld M, Senturk H, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: A randomised trial. Lancet 2005;365(9454):123–9.
17. Pas SD, Fries E, De Man RA, et al. Development of a quantitative real-time detection assay for hepatitis B virus DNA and comparison with two commercial assays. J Clin Microbiol 2000;38(8):2897–901.

18. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22(6):696–9.

19. Bortolotti F, Cadrobbi P, Crivellaro C, et al. Long-term outcome of chronic type B hepatitis in patients who acquire hepatitis B virus infection in childhood. Gastro enterology 1990;99(3):805–10.

20. Shiffman M, Marcellin P, Jeffers L, et al. HBsAg seroconversion in Adefovir Dipivoxil (ADV) treated chronic hepatitis B (CHB) patients. J Hepatol 2004;2004:17.

21. Lau GK, Piratvisuth T, Luo KX, et al. Peginterferon alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005;352(26):2682–95.

22. Maynard M, Parvaz P, Durantel S, et al. Sustained HBs seroconversion during lamivudine and adefovir dipivoxil combination therapy for lamivudine failure. J Hepatol 2005;42(2):279–81.

23. Guidotti LG, Rochford R, Chung J, et al. Viral clearance without destruction of infected cells during acute HBV infection. Science 1999;284(5415):825–9.

24. van Bommel F, Wunsche T, Mauss S, et al. Comparison of adefovir and tenofovir in the treatment of lamivudine-resistant hepatitis B virus infection. Hepatology 2004;40(6):1421–5.

25. van Nunen AB, Hansen BE, Suh DJ, et al. Durability of HBeAg seroconversion following antiviral therapy for chronic hepatitis B: Relation to type of therapy and pretreatment serum hepatitis B virus DNA and alanine aminotransferase. Gut 2003;52(3):420–4.

26. van Zonneveld M, Honkoop P, Hansen BE, et al. Longterm follow-up of alpha-interferon treatment of patients with chronic hepatitis B. Hepatology 2004;39(3):804–10.

27. Sanchez-Tapias JM, Costa J, Mas A, et al. Influence of hepatitis B virus genotype on the long-term outcome of chronic hepatitis B in western patients. Gastroenterol ogy 2002;123(6):1848–56.

28. Erhardt A, ReinekeU, Blondin D, et al. Mutations of the core promoter and re sponse to interferon treatment in chronic replicative hepatitis B. Hepatology 2000;31(3):716–25. HBsAg Loss After Pegylated Interferon Therapy 303

29. Chu CJ, Lok AS. Clinical significance of hepatitis B virus genotypes. Hepatology 2002;35(5):1274–6.

30. Chu CJ, Hussain M, Lok AS. Hepatitis B virus genotype B is associated with earlier HBeAg seroconversion compared with hepatitis B virus genotype C. Gastroen terology 2002;122(7):1756–62.

31. Kao JH, Wu NH, Chen PJ, et al. Hepatitis B genotypes and the response to inter feron therapy. J Hepatol 2000;33(6):998–1002.

32. Hou J, Schilling R, Janssen HLA, et al. Hepatitis B virus genotypes and response to interferon alpha treatment in chronic hepatitis B. Hepatology 2001;34(4): 447A.

33. Marcellin P, Lau GK, Bonino F, et al. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2004;351(12):1206–17.

34. Marcellin P, Martinot-Peignoux M, Loriot MA, et al. Persistence of hepatitis B virus DNA demonstrated by polymerase chain reaction in serum and liver after loss of HBsAg induced by antiviral

Chapter 5

Successful treatment with peginterferon alfa-2b of HBeAg-positive HBV non-responders to standard interferon or lamivudine

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ABSTRACT

Objectives: Antiviral therapy leads to HBeAg seroconversion in 10-40% of the patients with HBeAg-positive chronic hepatitis B. Non-response may result in progression of liver disease and increased risk of hepatocellular carcinoma. As part of a global randomized controlled trial we investigated the efficacy (i.e. loss of HBeAg at the end of follow-up) of peginterferon alfa-2b (Peg-IFN α-2b) in patients who failed to respond to previous courses of standard interferon (IFN) or lamivudine.

Methods: We analyzed a total of 76 previous non-responders, 37 were non-responders to standard IFN, 17 were non-responders to lamivudine, and 22 were non-responders to both therapies. All patients received a 52-week course of 100 µg Peg-IFN α-2b weekly combined with either 100 mg lamivudine daily or a placebo. After therapy patients were followed for 26 weeks.

Results: Thirteen (35%) non-responders to previous IFN, 5 (29%) non-responders to previous lamivudine, and 4 (22%) non-responders to both IFN and lamivudine responded to treatment with Peg-IFN α-2b. No difference in response was found for those treated with Peg-IFN α-2b alone or in combination with lamivudine. Non-responders to prior IFN therapy with baseline ALT > 4 x ULN responded better to Peg-IFN α-2b than those with ALT levels $\leq$ 4 x ULN (53% vs. 20%, respectively, $p = 0.036$).

Conclusions: Peg-IFN α-2b is effective in approximately one-third of patients who failed to respond to previous treatment with standard IFN or lamivudine. High serum ALT level at baseline of Peg-IFN α-2b therapy was the best predictor for response in these patients.

INTRODUCTION

Chronic infection with hepatitis B virus (HBV) affects approximately 400 million people worldwide[1,2], and is usually associated with continuing inflammatory activity and progression of liver disease, which in turn leads to increased risk of cirrhosis, decompensated liver disease and hepatocellular carcinoma [2,3].

Therapy with lamivudine establishes high initial response rates, but reappearance of wild-type HBV occurs in up to 50%-60% of patients after cessation of therapy[4]. Continuous treatment with nucleoside analogues has been associated with the emergence of mutations in the polymerase genome which cause viral resistance[5,6]. Standard interferon alfa (IFN) therapy has been shown to convert a state of active disease into inactive disease (HBeAg seroconversion) in about 20-30% of HBeAg-positive chronic hepatitis B patients[1,7].

For the growing population of chronic hepatitis B patients who do not exhibit a sustained response to antiviral therapy with IFN or lamivudine, it is important to assess whether retreatment with either IFN or peginterferon is effective. Studies of retreatment with conventional IFN or lamivudine in previous non-responders to IFN therapy yielded disappointing HBeAg clearance rates[8-11]. Recently, studies in HBeAg-positive patients showed efficacy in 30-40% after one year of treatment with peginterferon alone or in combination with lamivudine[12-14]. We studied the efficacy of peginterferon alfa-2b (Peg-IFN α-2b) in previous non-responders to conventional IFN or lamivudine treatment.

MATERIALS AND METHODS

Patients and methods

For this study, we extracted the data from a multicenter randomized controlled trial which compared Peg-IFN α-2b mono-therapy to Peg-IFN α-2b combination therapy with lamivudine for chronic hepatitis B[13]. Patients received in a 1:1 ratio 100 µg Peg-IFN α-2b weekly with placebo or with 100 mg lamivudine daily for 52 weeks. After 32 weeks the dose of Peg-IFN α-2b was halved into 50 µg in order to prevent side-effects and early treatment discontinuation. Follow-up after therapy lasted 26 weeks. The inclusion and exclusion criteria were reported previously[13]. Major inclusion criteria were: HBeAg-positive on two occasions within 8 weeks of randomization, elevated serum alanine aminotransferase (ALT) at least twice the upper limit of normal (ULN), and serum HBV DNA > 10^5 copies/mL.

The original study contained 266 patients[13] of whom 190 were treatment naive and 76 previously treated with IFN and or lamivudine. The analysis of the current study was based on the 76 patients who had previously received antiviral therapy. Among these, 37 were non-responders to standard IFN, 17 to lamivudine, and 22 to both IFN and lamivudine. Of these 22 patients, 12 had been treated sequentially and 10 had received combination therapy.

For patients with multiple previous therapies with IFN or lamivudine, only the last course was analyzed. Response to Peg-IFN α-2b therapy was defined as loss of HBeAg after follow-up.

During therapy and follow-up, patients were seen monthly for routine examination, as well as biochemical and hematological assessments. Transaminases were assessed locally and therefore expressed as times upper limits of normal (ULN). HBV DNA was also assessed monthly (detection limit 400 copies/ml) using in-house Taqman PCR based on the Eurohep standard[15]. HBeAg (AxSYM, Abbott) and HBsAg (AxSYM, Abbott) status were assessed at week 0, 32, 52 (during treatment) and week 78 (after follow-up). HBV genotype and YMDD mutation analysis were assessed by Inno-Lipa Assay (Innogenetics).

Statistical analysis

In order to establish predictors for response of the retreated, population the following variables were tested by univariate logistic regression analysis: baseline ALT, HBV DNA, age, sex, weight, race, HBV genotype and mode of transmission. Characteristics of previous treatment with IFN, i.e. cumulative dose, duration of therapy, time between end of previous therapy and start of new therapy, and type of IFN, were also tested. All variables were checked for interaction with previous therapy. Those variables with p-value < 0.05 were retained in the final multivariate logistic regression model. In order to calculate compliance with therapy, survival analysis by means of the log rank test was performed.

RESULTS

Baseline characteristics of previous non-responders are given in Table 1. Median treatment duration was 24 weeks (range 8 - 52) for previous standard IFN (median dose 18 MU/week), and 54 weeks (range 2 - 124) for previous treatment with lamivudine (dose 100mg/day). Median treatment duration for previous non-responders to both IFN and lamivudine was 24 weeks (range 3-48) for IFN and 52 weeks (range 4-411) for lamivudine. Median interval to retreatment was 132 weeks (range 26 - 541) for previous IFN and 61 weeks (range 26 - 150) for previous lamivudine. Median interval to retreatment for non-responders to both therapies was 111 weeks (range 32-576) and 42 weeks (range 27-366) for IFN and lamivudine, respectively. A total of 11 patients had a YMDD-mutant at the start of Peg-IFN α-2b therapy. The most prevalent HBV genotypes were genotype A and D.

Table 1. Data on previous non-responders to standard IFN, lamivudine, and IFN and lamivudine therapy

Characteristics	treatment naives N = 190	non-response to IFN N = 37	non-response to lamivudine N = 17	non-response to IFN and lamivudine N = 22
Age*	33.1 ± 12.4	39.1 ± 13.3	38.3 ± 13.2	41.2 ± 13.0
Male (%)	140 (74)	33 (89)	13 (77)	19 (86)
Race (%)				
Caucasian	131 (70)	35 (95)	9 (53)	21 (96)
Asian/mongoloid	43 (23)	2 (5)	7 (41)	0
ALT* (x ULN)	4.1 ± 3.5	4.6 ± 3.3	3.7 ± 2.3	5.4 ± 3.9
Log HBV DNA*	9.0 ± 1.0	8.9 ± 0.9	9.3 ± 0.5	9.4 ± 0.8
HBV genotype (%)				
A	59 (31)	23 (62)	4 (24)	4 (18)
B	16 (8)	1 (3)	6 (35)	0
C	36 (19)	1 (3)	2 (12)	0
D	73 (38)	9 (24)	4 (24)	17 (77)
Previous dose IFN†	-	18 (4.5 –35) MU/wk	-	18 (5 – 30) MU/wk
Previous duration IFN†	-	24 (8-52) wks	-	24 (3-48) wks
Interval to retreatment with IFN†	-	132 (26-541) wks	-	111 (32-576) wks
Previous dose lamivudine	-	-	100 mg/d	100 mg/d
Previous duration lamivudine†	-	-	54 (2-124) wks	52 (4-411) wks
Interval to retreatment with lamivudine†	-	-	61 (24-150) wks	42 (27-366) wks
YMDD mutation (%)	-	-	4 (24)	7 (32)

*Mean ± SD † Median (range)

Loss of HBeAg, HBV DNA response and ALT normalization

The rate of serum HBeAg clearance (response), HBV DNA negativity (< 400 copies/mL) and ALT normalization according to the treatment regimen are given in Table 2. Although patients with prior exposure to lamivudine tended to respond better to Peg-IFN α–2b monotherapy, response rates for Peg-IFN α-2b monotherapy and combination therapy were in general comparable. Therefore, further analysis was performed for both treatment groups together. Loss of HBeAg at the end of follow-up occurred in 13 of non-responders to IFN (35%), 5 of non-responders to lamivudine (29%), and 4 of non-responders to IFN and lamivudine (18%) (Figure 1). Among the non-responders to IFN, 11 of the 13 with HBeAg loss developed anti-HBe (85%); all responders of the previous lamivudine group and in the previous IFN and lamivudine group developed anti-HBe. Among all prior non-responders HBsAg loss occurred only once (1%; non-responders to lamivudine). In comparison, HBsAg loss occurred in 17 of the treatment-naive patients (9%), (p = 0.03). HBV DNA negativity was found in 2 (5%) of the previous IFN non-responders and in 1 (5%) of the lamivudine non-responders. Normalization of ALT occurred in 9 non-responders to IFN (24%), 7 non-responders to lamivudine (41%), 3 of non-responders to IFN and lamivudine (14%), and 71 (37%) naïve patients. Of the 11 patients with a YMDD at start of therapy, 2 (18%) were HBeAg-negative at the start end of follow-up.

Table 2. HBeAg clearance, ALT normalization and HBV DNA < 400 copies/mL at the end of follow-up according to previous treatment

	Peg-IFN α2b Lamivudine	Peg-IFN α2b Placebo	P value
HBeAg clearance			
Naive	35/92 (38%)	38/98 (39%)	0.91
Previous IFN	8/19 (42%)	5/18 (28%)	0.36
Previous LAM	1/9 (11%)	4/8 (50%)	0.13
Previous IFN and LAM	2/10 (20%)	2/12 (17%)	1.0
ALT normalization			
Naive	34/85 (40%)	37/88 (42%)	0.79
Previous IFN	5/19 (26%)	4/18 (22%)	1.0
Previous LAM	4/9 (44%)	3/8 (47%)	1.0
Previous IFN and LAM	3/10 (30%)	0/12 (0%)	0.08
HBV DNA < 400 copies/ml			
Naive	9/83 (11%)	9/87 (10%)	0.91
Previous IFN	2/17 (12%)	0/14 (0%)	0.49
Previous LAM	1/7 (14%)	0/8 (0%)	0.47
Previous IFN and LAM	0/9 (0%)	0/8 (0%)	NA

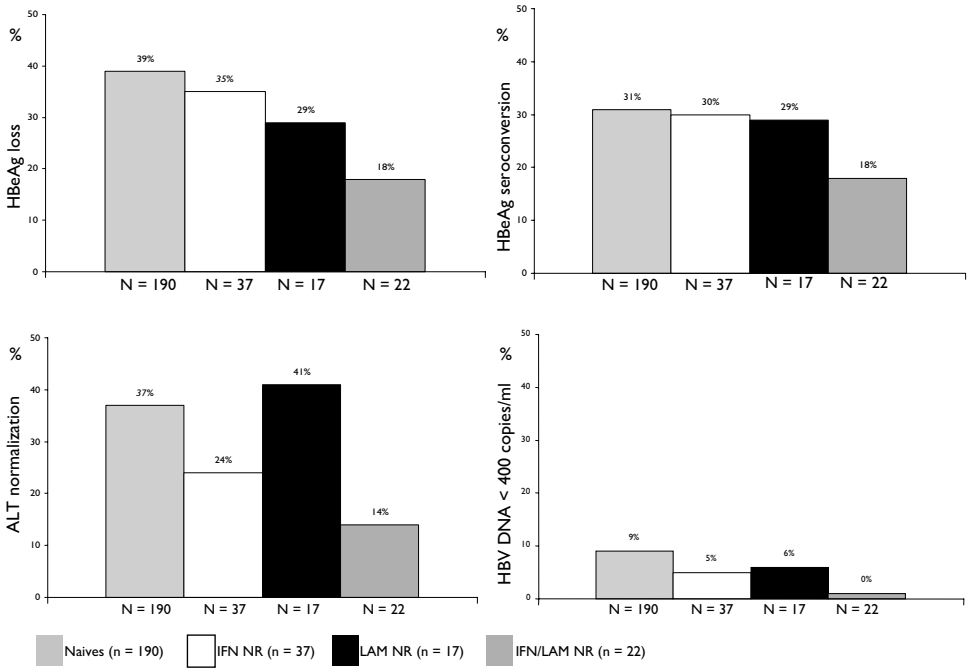

Figure 1. HBeAg loss, HBeAg seroconversion, ALT normalization, and HBV DNA response in naive patients (n=190), IFN nonresponders (n=37), lamivudine non-responders (n=17), and non-responders to both IFN and lamivudine (n=22) at the end of follow-up. HBeAg loss and ALT normalization: naive versus non-responders to both IFN and LAM, p = 0.07 and p = 0.02, respectively.

Response prediction

In order to be able to determine predictors for response (loss of HBeAg at the end of follow-up), we performed logistic regression analysis for the combined group of previous non-responders (n=76). Among all prior non-responders, the baseline ALT level was the only significant parameter predicting response (Table 3). Treatment allocation, baseline HBV DNA level and HBV genotype were not associated with response of previous non-responders. Multivariate analysis confirmed higher baseline ALT levels as an independent predictor for response. Figure 2 shows the response probability for all groups. In particular non-responders to prior IFN therapy with baseline ALT above 4 x ULN (n=17) responded better to Peg-IFN α–2b therapy than those with ALT levels below 4 x ULN (n=20; 53% vs. 20%, respectively, p = 0.036). Among non-responders to previous lamivudine, patients with ALT above 4 x ULN responded in 50% vs. 18% of patients with baseline ALT below 4 x ULN (p = 0.28); among non-responders to IFN and lamivudine, corresponding response rates were 30% and 8%, (p = 0.29), respectively.

Table 3. Univariate analysis predicting response of all previous non-responders (N = 76)

		95% CI		
Baseline	hazard ratio	lower bound	upper bound	p-value
Age per 10 yrs	0.89	0.60	1.31	0.55
Weight per 10 kg	1.10	0.74	3.11	0.66
ALT (x ULN)*	**1.27**	**1.08**	**1.51**	**0.005**
ALT (x ULN) > 4	**4.55**	**1.51**	**13.5**	**0.006**
Log HBV DNA**	0.90	0.48	1.69	0.74
Log HBV DNA > 9	0.75	0.27	2.13	0.59
HBV genotype				
A	1.0			
B	1.9	0.25	13.9	0.54
C	0.002	0.000	-	0.76
D	0.91	0.26	3.24	0.88
Other	1.53	0.21	11.0	0.67
Combination Rx †	1.03	0.62	1.71	0.90
Previous duration Lamivudine	0.99	0.96	1.01	0.29
Previous duration IFN	1.01	0.94	1.08	0.92
Previous dose IFN	1.0	0.99	1.0	0.32

**RR per increase of 1 × ULN; † RR per increase of 1 log;

‡ 100 µg Peg-IFN α-2b + 100 mg lamivudine.

All variables were checked for interaction with previous treatment.

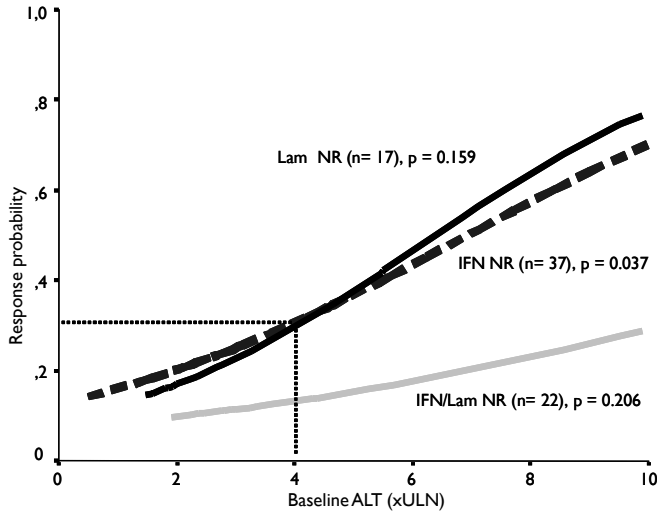

Figure 2. Baseline ALT in serum and probability of response. Response to Peg-IFN α-2b among non-responder (NR) increases with higher baseline ALT levels. In particular, ALT baseline levels above 4 xULN improve probability of response. Baseline ALT levels are less predictive for response in previous non-responders to both IFN and lamivudine.

Safety and adherence

The frequency of adverse events for this population, irrespective of exposure to previous antiviral therapy, has been described elsewhere[13]. Anorexia was seen more often in naive patients (19%) than in the retreated patients (9%, p = 0.05). Occurrence of all other adverse events was comparable for the retreated and the naive population. Among previous non-responders, local reaction at the Peg-IFN α-2b injection site was found for 19 of 59 (32%) non-responders to an IFN-based regimen versus 1 of 17 (6%) non-responders to lamivudine alone (p = 0.03). Overall, treatment with Peg-IFN α-2b was discontinued early in 24 cases (9%). Thirteen were naive patients (7%) and 11 retreated patients (14%; p = 0.05; Figure 3). Also the frequency of Peg-IFN α-2b dose reduction was higher, although not significant, in retreated patients (Figure 3). Neutropenia was the most frequent cause for dose reduction. Among the previous non-responder groups, no differences in early treatment discontinuation were found: 8 non-responders to an IFN-based regimen (14%) and 3 non-responders to lamivudine alone (18%, p = 0.7) stopped treatment early.

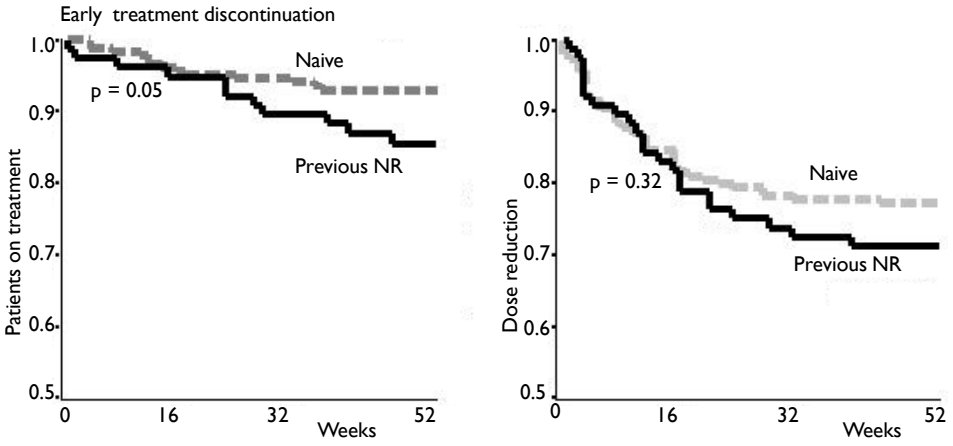

Figure 3. Early treatment discontinuation and dose reduction of Peg-IFN α-2b therapy in naive and retreated patients. Early treatment discontinuation was seen more often among retreated patients (p = 0.05). A similar trend was found for dose reduction of Peg-IFN α-2b therapy (p = 0.32).

DISCUSSION

The efficacy of Peg-IFN α-2b alone or in combination with lamivudine in previous non-responders to standard IFN or lamivudine therapy has not been investigated to date. In the current study, we found loss of HBeAg in 35% of non-responders to prior IFN, 29% of non-responders to prior lamivudine, and 18% of non-responders to prior IFN and lamivudine. Combination of Peg-IFN α-2b and lamivudine did not lead to higher response rates at the

end of follow-up than Peg-IFN α-2b alone for any of the previous non-responder groups. Compliance with Peg-IFN α-2b therapy among patients previously treated with IFN or lamivudine was high, but early treatment discontinuation was more frequent among retreated than naive patients. The safety profile was comparable for naive and retreated patients.

In the past, repeated use of standard lymphoblastoid IFN alfa treatment for 4 months led to loss of HBeAg in only 11% of our patients[9]. The higher response in our present study could be explained not only by the use of peginterferon, but also by the fact that our previous study consisted of a difficult-to-treat population with expected low rates of HBeAg loss based on a response prediction model[9]. In the current study patients were treated with peginterferon instead of conventional interferon, which could explain the improved efficacy. Patients also underwent a longer treatment duration. It may be possible that longer treatment duration also effected the positive outcome, but we cannot confirm this in the current study. Another retreatment study in which a 6 months course with standard IFN alfa 2a was followed by 6 month of follow-up showed HBeAg clearance in 41% of patients (8). Baseline characteristics to predict treatment response were probably favorable in this study since the untreated control group exhibited a high HBeAg response rate of 17%.

Lau and colleagues recently reported on the use of peginterferon alfa 2a alone or combined with lamivudine for HBeAg-positive patients previously exposed to IFN or lamivudine[16]. Patients were treated for 48 weeks and assessed 24 weeks after the end of treatment. Treatment with peginterferon alfa 2a alone resulted in an HBeAg seroconversion rate of 43% for previous IFN non-responders and 32% for previous lamivudine non-responders. For patients treated with the combination these HBeAg seroconversion rates were 34% and 25%, respectively. Comparison of these response rates with those found in our study is difficult since the baseline characteristics were not comparable. Furthermore, for the peginterferon alfa 2a study, data on dose and duration of previous IFN and lamivudine exposure as well as the interval between previous therapy and peginterferon alfa 2a therapy, are not yet available.

In the current study, adding lamivudine to Peg-IFN α-2b did not enhance the response rate for either treatment-naives or previous non-responders. The efficacy of lamivudine for previous non-responders to IFN has been poorly examined so far. A study by Schiff et al. reported an HBeAg clearance rate of 32% after 52 weeks of lamivudine and 16 weeks of post-treatment follow-up[11]. This response rate is within the same range as the 35% HBeAg loss 26 weeks after combination therapy for IFN non-responders in our study. Nevertheless, the off-treatment sustainability of both HBV DNA and HBeAg response after lamivudine monotherapy has been debated and long-term follow-up studies are necessary[4, 17]. The recent study by Lau et al. with 24 weeks of follow-up after one year of lamivudine showed a sustained HBeAg seroconversion response of only 13%[16].

In an attempt to predict which previous non-responders benefit most from Peg-IFN α-2b treatment, we performed a multivariate logistic regression analysis. Among the previous non-responders to IFN, response to therapy with Peg-IFN α-2b was significantly higher for patients with baseline ALT levels above 4 x ULN compared to lower than 4 x ULN. A similar trend was found for non-responders to lamivudine. An approach for repeated therapy is to follow the patient until the moment when ALT values are above the level of 4 x ULN; initiating Peg-IFN α-2b therapy may then result in approximately 50% HBeAg response rates. Our analysis did not show duration and dose of previous therapy as predictors for response in our study, nor did HBV genotype or baseline HBV DNA level significantly influence response. In the original study HBV genotype and baseline HBV DNA level were important predictors for response[13], but not in the current study. Since our patient population consisted previous non-responders - even with a more favorable genotype or previous HBV DNA level - the positive predicting effect of these variables probably fainted. In addition, the majority of the present population had very high HBV DNA levels which could explain that HBV DNA at baseline did not influence the outcome. The importance of inflammatory activity rather than viral factors suggests that the actual state of the host immune reactivity is important to achieve response with Peg-IFN α-2b. The limited HBsAg clearance after (Peg)-IFN therapy among our non-responders and those studied previously[8, 11] further underlines the concept that the non-responder population may be predominantly in some state of immune tolerance and only transition to pre-treatment immune reactivity predisposes patients to undergo HBeAg clearance during Peg-IFN α-2b therapy.

In conclusion, Peg-IFN α-2b is effective in approximately one-third of previous non-responders to IFN or lamivudine therapy. Patients with prior exposure to IFN could be at risk for early Peg-IFN α-2b treatment discontinuation. High baseline ALT levels are the best predictors for response. Particularly, in previous non-responders with ALT levels above 4 x ULN, Peg-IFN α-2b therapy should be considered.

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REFERENCES

1. Wong DK, Cheung AM, O'Rourke K, et al. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. Ann Intern Med. 1993 Aug 15;119(4):312-23.
2. Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000--summary of a workshop. Gastroenterology. 2001 Jun;120(7):1828-53.
3. Beasley RP. Hepatitis B virus. The major etiology of hepatocellular carcinoma. Cancer. 1988 May 15;61(10):1942-56.
4. van Nunen AB, Hansen BE, Suh DJ, et al. Durability of HBeAg seroconversion following antiviral therapy for chronic hepatitis B: relation to type of therapy and pretreatment serum hepatitis B virus DNA and alanine aminotransferase. Gut. 2003 Mar;52(3):420-4.
5. Honkoop P, Niesters HG, de Man RA, et al. Lamivudine resistance in immunocom petent chronic hepatitis B. Incidence and patterns. J Hepatol. 1997 Jul;26(6):1393-5.
6. Dienstag JL, Goldin RD, Heathcote EJ, et al. Histological outcome during long-term lamivudine therapy. Gastroenterology. 2003 Feb;124(1):105-17.
7. Schalm SW, Heathcote J, Cianciara J, et al. Lamivudine and alpha interferon combination treatment of patients with chronic hepatitis B infection: a randomised trial. Gut. 2000 Apr;46(4):562-8.
8. Carreno V, Marcellin P, Hadziyannis S, et al. Retreatment of chronic hepatitis B e antigen-positive patients with recombinant interferon alpha-2a. The European Concerted Action on Viral Hepatitis (EUROHEP). Hepatology. 1999 Aug;30(1):277-82.
9. Janssen HL, Schalm SW, Berk L, et al. Repeated courses of alpha-interferon for treatment of chronic hepatitis type B. J Hepatol. 1993;17 Suppl 3:S47-51.
10. Munoz R, Castellano G, Fernandez I, et al. A pilot study of beta-interferon for treatment of patients with chronic hepatitis B who failed to respond to alpha-interferon. J Hepatol. 2002 Nov;37(5):655-9.
11. Schiff ER, Dienstag JL, Karayalcin S, et al. Lamivudine and 24 weeks of lamivudine/ interferon combination therapy for hepatitis B e antigen-positive chronic hepatitis B in interferon nonresponders. J Hepatol. 2003 Jun;38(6):818-26.
12. Cooksley WG, Piratvisuth T, Lee SD, et al. Peginterferon alfa-2a (40 kDa): an ad vance in the treatment of hepatitis B e antigen-positive chronic hepatitis B. J Viral Hepat. 2003 Aug;10(4):298-305.
13. Janssen H, Van Zonneveld M, Senturk H, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine as the treatment for HBeAg-positive chronic hepatitis B. Lancet. 2005;365:123-29.
14. Lau GK, Piratvisuth T, Luo KX, et al. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med. 2005 Jun 30;352(26):2682-95.

15. Pas SD, Fries E, De Man RA, et al. Development of a quantitative real-time detection assay for hepatitis B virus DNA and comparison with two commercial assays. J Clin Microbiol. 2000;38(8):2897-901.

16. Lau GKK, Piratvisuth T, Luo K-X, et al. Peginterferon alfa-2a (40kDa)(Pegasys) versus Peginterferon alfa-2a plus lamivudine versus lamivudine in HBeAg-positive chronic HBV: effect of previous treatment and drug exposure on sustained response. J Hepatol. 2005;42(Suppl 2):15.

17. Rizzetto M, Tassopoulos NC, Goldin RD, et al. Extended lamivudine treatment in patients with HBeAg-negative chronic hepatitis B.
J Hepatol. 2005 Feb;42(2):173-9.

Chapter 6

The effect of pegylated interferon-α on the treatment of lamivudine resistant chronic HBeAg positive hepatitis B virus infection

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ABSTRACT

Background/Aims: To determine the response to pegylated interferon-α treatment of HBeAg-positive hepatitis B patients with proven lamivudine resistance.

Methods: Sixteen HBeAg-positive HBV patients with YMDD mutations were treated with pegylated interferon. Median treatment duration was 52 weeks (range 20–53), with a 26-week follow-up.

Results: Two of 16 (12.5%) patients seroconverted to HBeAg negative and achieved sustained virological (HBV-DNA levels below ^{10}log5 copies/ml) together with biochemical (normalization of serum ALT levels) responses. Compared with the strong signal in all other patients, only these two patients had a faint signal in the lamivudine resistance assay. For all patients, the median viral load decreased from ^{10}log 9.4 to 7.9 copies/ml ($p = 0.001$) during treatment but rebounded to a median of ^{10}log 8.7 copies/ml after treatment cessation. Similarly, elevated median ALT levels at baseline decreased with treatment but rebounded after the end of treatment.

Conclusions: In the largest cohort study to date, pegylated interferon-α therapy showed marginal efficacy in the presence of lamivudine resistance but such therapy may be beneficial in patients with only small amounts of mutant virus. In our opinion, an analysis of the patient subgroup harbouring an YMDD-mutation should be included in all future studies of pegylated interferon-α in chronic hepatitis B.

INTRODUCTION

Although 350–400 million people worldwide are affected by hepatitis B, to date, treatment for patients is frequently unsuccessful. Chronic hepatitis B is an immunological- based liver disorder, and there is increasing evidence that only a complete and vigorous HBV-specific immune response can achieve control and elimination of the virus, preventing disease progression[1]. The subgroup of HBeAg-positive hepatitis B patients with proven lamivudine resistance are little studied, and existing studies are often hampered by their extent and design[2–5], which makes it difficult to draw definitive conclusions about the optimal treatment for this group. In the study reported here, which is the largest cohort study to date, we investigate the response of HBeAg-positive hepatitis B patients with proven lamivudine resistance to pegylated interferon-α.

The nucleoside analogue lamivudine is an effective inhibitor of viral DNA polymerase. It suppresses replication of HBV, improving transaminase levels and liver histology and enhancing the loss of hepatitis B e-antigen (HBeAg)[6,7]. However, sustained response after discontinuation of treatment occurs in only 10–15% of lamivudine-treated patients [8]. Another drawback is the emergence of mutations, in the tyrosine–methionine–aspartate–aspartate (YMDD-motif) of the viral polymerase, which are associated with resistance[9]. Mutations sometimes arise as little as 6 months after initiation of treatment[5] with a resistance rate of 15–30% after 1 year, increasing to approximately 60–70% after 4 years of continuous treatment [6,7,10–13]. Some patients who experience virological breakthrough may develop acute exacerbation, leading to liver decompensation and death[9].

European guidelines recommend pegylated interferon-α as first-line treatment for both HBeAg-positive and -negative patients[13] but there remain several unanswered questions related to its uptake as a panacea treatment for hepatitis B. There is conflicting evidence on the effect lamivudine resistance, caused by mutations in the YMDDmotif, on the outcome of pegylated interferon-a therapy. Recently, our department coordinated a large, independent, randomised, double-blind multicentre trial to determine the effects of pegylated interferon-a treatment in HBeAg-positive patients either alone or in combination with lamivudine[14]. The presence of the complete data set and patient samples from this trial enabled us to devise this retrospective cohort study of the outcome of pegylated interferon-a treatment in HBeAg-positive patients carrying the YMDD-motif mutated virus, which is reported here.

PATIENTS AND METHODS

Study design

In this retrospective, comparative, cohort study, data were compiled from the patient files and virological records of a large, multicentre trial previously conducted in our department in which the efficacy of pegylated interferon α-2b, either alone or in combination with lamivudine, was compared in a randomised trial of chronic hepatitis B patients[14]. In addition, patients treated by the same protocol outside this study were also included.

Inclusion and exclusion criteria

Eligible patients were HBeAg-positive with resistance to lamivudine as a result of lamivudine treatment before the start of interferon therapy. Resistance was confirmed by detection of a mutation in the YMDD motif of the RNA-dependent DNA polymerase gene of the virus. All patients with lamivudine-resistant virus were included in this analysis, regardless of differences in the subsequent interferon therapy (mono- or combination therapy). If mutational data were not available on record, retrospective analysis was carried out on the corresponding stored serum samples. Where a time point was missing from the records, results from the nearest date of sampling were taken, within an interval of 4 weeks. Patients were excluded from the study if they were receiving antiviral treatment at the time of enrolment in the original study.

Treatment and outcome measures

All patients were treated with pegylated interferon α-2b either in monotherapy (100 µg/week) or in combination with lamivudine (lamivudine 100 mg/day) for more than 20 weeks and were followed-up for at least a further 16 weeks post-therapy. During treatment and follow-up, patients attended outpatient clinics every 4 weeks for routine examination and laboratory tests. Assessments were made at baseline, Weeks 16, 32 and 52, and after 26 weeks of followup, as appropriate. Outcome measures were assessed at the end of treatment (Week 52) and at the end of follow-up (Week 26). The primary outcome measure was loss of HBeAg from serum. Secondary outcomes were return to normal of serum ALT levels, concentrations of HBV DNA below 200,000 copies/mL and concentrations of HBV DNA below the level of detection of the assay (Taqmanw assay; 400 copies/mL).

Biochemical and virological assessments

Viral load was determined by HBV DNA serum levels and seroconversion by the presence of HBeAg or anti-HBe. HBeAg and anti-HBe concentrations were determined using a Microparticle Enzyme Immune Assay (MEIA, Abbott, Chigaco, IL). During treatment with interferon, HBV-DNA serum levels were determined by an in-house qPCR (Taqmanw assay)

calibrated using Eurohep HBV DNA standards[15]. Used quantitatively, the Taqman assay enables accurate determination to levels of 1000 copies/mL[16]. HBV genotypes and mutation analysis of the YMDD motif at the rtM204M side of the viral polymerase gene were determined using the Inno-Lipa assay (Innogenetics Ghent, Belgium). The extent of liver inflammation was determined by measuring serum alanine aminotransferase (ALT) levels. To correct for the heterogeneity of local assays, ALT levels are expressed as values representing a ratio to local upper limit of normal (xULN) and shown as medians with their range.

Statistical analysis

Continuous variables are expressed as median with their range. Median scores were compared by Wilcoxon Signed-Rank Test. The Mann–Whitney test was used for the comparison of groups. A two-tailed p-value of < 0.05 was considered statistically significant. All analyses used SPSS (version 12.0.1; Chicago, IL, USA). Subgroup analyses were performed to determine whether known predictors of response to therapy accounted for response to pegylated interferon treatment in this study. The most frequently cited predictors of response include; previous interferon-alfa treatment, genotype, high ALT levels and low viral load[13,14].

RESULTS

Sixteen HBeAg-positive patients fulfilled the study criteria. The baseline characteristics of the patients are shown in Table 1. Of the patients in this analysis, the majority (12/16) received pegylated interferon-α treatment monotherapy and the remaining four were treated with the same weekly dose of pegylated interferon together with 100 mg of lamivudine per day (combination therapy). Fifteen of the 16 patients received treatment for 52 weeks and, for 13 patients, all data (ALT, HBV-DNA and e-status) were available at 26 weeks of follow-up. In viral samples from two patients only faint mutation bands were visible by the Inno-Lipa Assay.

HBeAg-status

Two of 16 patients (12.5%; 95% CI—6.0 to 31%) seroconverted to HBeAg negative, and these two patients also had a sustained virological and biochemical response (HBV-DNA levels less than 10^5 copies/ml and normal ALT at 26 weeks follow up)

Table 1. Baseline characteristics

	Total	Peginterferon monotherapy	Peginterferon + lamivudine combination therapy
Male/ female gender (*)	14 / 2	10 / 2	4 / 0
Median age (range)	41.5 (25-72)	41.5 (27-72)	41.5 (25-48)
Race (caucasian / asian)(*)	14 / 2	11 / 1	3 / 1
Previous IFN treatment (yes/no;*)	7 / 9	5 / 7	2 / 2
Median time start Peg-IFN after lamivudine (weeks) (range)	42.3 (5.3-176.1)	39.9 (5.3-176.1)	64.6 (31.0-100.6)
Fibrosis according Ishak no. 0 / 1 / 2 / 3 / 4 / 5 / 6 // missing (*)	0 / 3 / 1 / 6 / 0 / 1 / 1 // 4	0 / 2 / 1 / 5 / 0 / 0 / 1 // 3	0 / 1 / 0 / 1 / 0 / 1 / 0 // 1
Median ALT x ULN	3.5 (1.5-11.0)	3.6 (1.5-11.0)	3.5 (1.7-7.2)
Median log_{10} HBV-DNA	9.4 (8.7-10.4)	9.3 (8.7-10.4)	9.8 (9.1-10.2)
Genotype A/D/other (*)	6 / 6 / 4	5 / 5 / 2	1 / 1 / 2
YMDD mutants V / M+I / M+V / M+V+I (*)	1 / 4 / 6 / 5	1 / 2 / 4 / 5	0 / 2 / 2 / 0

* number of patients. Baseline characteristics for the total group and the subgroups Peginterferon monotherapy and peginterferon + lamivudine combination therapy.

Table 2. Course of liver inflammation and viral load

	baseline		Week 52		Week 26 follow-up	
	ALT	HBV DNA	ALT	HBV DNA	ALT	HBV DNA
Total n=16	3.5 (1.5-11.0)	9.4 (8.7-10.4)	1.3 (0.9-11.6)[1]	7.9 (1.7-10.0)[1]	2.3 (0.9-5.1)[1]	8.7 (2.6-10.3)[1]
Monotherapy n=12	3.4 (1.5-11.0)	9.3 (8.7-10.4)	1.4 (0.9-11.6)	7.9 (4.3-9.5)[1]	2.5 (1.5-5.1)	8.7 (8.1-10.3)[1]
Combination therapy n=4	3.5 (1.7-7.2)	9.8 (9.1-10.2)	1.3 (1.1-2.5)	4.2 (1.7-10.0)	1.0 (0.9-3.1)[2]	6.7 (2.6-10.1)

The course of liver inflammation as measured as ratio of ALT as upper limit of normal and viral load (10log copies/ml) at baseline, end of treatment (week 52)and 26 week of follow-up for the total group and the subgroups receiving Peginterferon-α monotherapy or Peginterferon-α + lamivudine combination therapy. Number in superscript (1) indicate a significant difference ($P < 0.05$) compared to the baseline value (1). Number in superscript (2) indicate a significant difference ($P = 0.05$) between two groups.

Viral load

The median viral load for the whole patient group decreased by 1.5 ^{10}log copies/ml to 7.9 (range 1.7–10.0) during the treatment period (p = 0.001). However, this reduction was not sustained and by 26 weeks after treatment cessation the viral load rebounded to a median of 8.7 ^{10}log copies/ml (range 2.6–10.3) (Table 2).

Response assessed by ALT measurements

At baseline all patients had elevated ALT levels and a high viral load Ttable 1). By Week 52, ALT levels decreased to a median of 1.3 xULN (range 0.9–11.6), which is significant compared with baseline (p = 0.047)(Table 2).

After discontinuation of treatment, ALT levels increased to a median of 2.3 xULN (range 0.9–5.1) by 26-weeks of follow-up. Only 3 (19%) patients had sustainable normal ALT levels.

Sustained response

By 26 weeks of follow up only 2 of 16 (12.5%; 95% CI- 6.0 to 31%) patients could be considered as sustained responders to treatment with pegylated interferon alfa by our primary outcome measure, and only 2/13 (15.4%) patients had viral load below 10^5 copies/mL. Only one responder had a HBV DNA less than 400 copies/mL at the end of follow-up. It is of note that the two responders were the patients where only very faint mutation bands were visible in the initial Inno-Lipa assay. Both responders were 48 years of age, an Asian male, interferon naive and a Caucasian male who had received prior interferon-a therapy. Time elapsed after discontinuation of lamivudine was 64.5 and 31.0 weeks and fibrosis according Ishak was 5 and 1, respectively. One patient had genotype B and the other genotype A. Both harboured the M552M + M552I mutation. ALT levels were 7.2 and 4.2 times elevated and HBV DNA levels were 9.1 and 9.7 ^{10}log copies/ml, respectively. None of the baseline characteristics was found to be significantly different compared to the nonresponders. One other patient had a normal ALT level after 26 weeks of follow up, but no HBe-seroconversion and HBV DNA above 10^5 copies/mL.

Monotherapy or combination therapy

The majority of the patients 12/16 (75%) were treated with pegylated interferon α-2b monotherapy. The baseline characteristics of these patients did not differ significantly from those receiving combination therapy (Table 1).

After 52 weeks of treatment the ALT levels had decreased equally in both groups (p = 0.791). In the combination therapy group, ALT levels continued to decrease during follow-up and the median ALT level was normal at end of follow-up which was significantly lower (p = 0.05) (Table 2). For the monotherapy group, despite achieving similar ALT levels to the

combination group at week 52, at the end of follow up the ALT levels increased to 2.5 xULN (Table 2.) Although the decrease in viral replication was more marked in the group receiving lamivudine in addition to interferon (5.6 ^{10}log compared with 1.4 ^{10}log copies/mL) during treatment, by 26-weeks after therapy overall viral levels for both groups were not significantly different ($p = 0.643$) (Table 2).

None of the 12 (0%) patients receiving pegylated interferon α-2b monotherapy had e-loss, normal ALT level or HBV DNA level below 10^5 copies/mL at the end of follow-up. Both responders were in the group that received combination therapy.

DISCUSSION

This is the largest study to date on the response to pegylated interferon-α treatment of HBeAg-positive hepatitis B patients who have YMDD-mutated virus after previous lamivudine treatment. Previous studies with this patient group used non-pegylated interferon-α and were limited in the number of patients studied[2–4], the length of treatment (6 months) and treatment schedules. The small number of responders to treatment prevented us from drawing definitive conclusions about the benefits of interferon therapy in this group. In contrast to the previous studies, in our larger study, all patients were treated with pegylated interferon-a for 52 weeks, in accordance with present guidelines and recommendations[13,17]. In our group of 16 patients, 2 (12.5%) responded positively to pegylated interferon-α treatment with HBeseroconversion, a drop in viral load and normalization of ALT levels. This response is lower than that observed in other trials of pegylated interferon-a treatment for HBeAg-positive patients, where the percentage of e-loss was over 30%[14,18]. However, the response rate was in line with that observed in the earlier trials with lamivudine-resistant patients, where between 16 and 22% of patients responded to pegylated interferon-α[2–5]. If we limit the analysis to the patients receiving monotherapy peginterferon-α none of the 12 (0%) responded to therapy.

There are several proposed predictors of response to treatment with interferon, including; previous interferon-a treatment, genotype, high ALT levels and low viral load[13,14]. In this small study we performed a sub-group analysis to determine which, if any, could explain the observed marked lack of response to pegylated interferon-α. None of these predictors were found to influence treatment outcome. We previously found that rate of e-loss was 25% in patients with genotype D virus, compared with 47% in those with genotype A[19]. Other studies concur that genotypes C and D are less responsive to interferon-α treatment compared with genotypes A and B[20–23]. A large randomised trial with pegylated interferon α-2a found no significant difference for response according to genotype; however, a trend for higher responses in patients with genotype A was observed[18]. In our study of patients

carrying YMDD-mutated virus, the relationship between genotype and treatment response was unclear. Both responders had genotypes that respond more favourably to treatment (genotypes A and B).

In our study it was striking that all patients with clear lamivudine resistance were unresponsive to interferon therapy. Notwithstanding the possibility that other unidentified factors may have influenced outcome, and although immunomodulatory therapy has not previously been linked to therapy failure with nucleoside analogues[24], the findings in our study may suggest that YMDD mutation impairs the immune response to HBV and reduces the efficacy of pegylated interferon-α treatment. In support of our putative explanation, both responders had, in contrast to the other subjects, very faint bands in the Inno-LiPA assay, which has a detection limit of about 5%. This assay was not formally quantified but this qualitative assay suggests that these patients had only a low quantity of mutant virus. Our results might suggest that, for subjects with emerging lamivudine resistance, early pegylated interferon-a therapy may be beneficial. Alternatively, other nucleoside analogues, such as adefovir, tenofovir and entecavir, which have been shown to be effective against lamivudine-resistant virus, may present a treatment option for this patient group[25–27].

Although suggestive of the negative impact of the presence of the YMDD-mutation on pegylated interferon α treatment, this study is too small to yield definitive results. More data are needed to further determine the effect of the YMDD-mutation on the efficacy of pegylated interferon-a treatment. Whether peginterferon-α combination therapy with other nucleoside/nucleotide analogues is beneficial for patients with lamivudine resistance has to be determined. This study is too small to make definite conclusions. The timing of the therapy may be of importance as both responders receiving combination therapy had only ittle amounts of mutant virus present in a sensitive assay. In our opinion, an analysis of the patient subgroup harbouring an YMDD-mutation should be included in all future studies of peginterferon in chronic hepatitis B.

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REFERENCES

1. Chisari FV, Ferrari C. Hepatitis B virus immunopathogenesis. Annu Rev Immunol 1995;13:29–60.
2. Pariente A, Tachet A, Poveda JD. Treatment of lamiduvine-resistant mutant chronic hepatitis B with interferon alpha. Gastroenterol Clin Biol 2002;26:1059–1060.
3. Danalioglu A, Kaymakoglu S, Cakaloglu Y, Demir K, Karaca C, Durakoglu Z, et al. Efficacy of alpha interferon therapy for lamivudine resistance in chronic hepatitis B. Int J Clin Pract 2004;58:659–661.
4. Ersoz G, Akarca US, Gunsar F, Karasu Z, Batur Y. Interferon retreatment for resistance to lamivudine plus interferon treatment. Turk J Gastroenterol 2003;14:223–227.
5. Suzuki F, Tsubota A, Akuta N, Someya T, Kobayashi M, Suzuki Y, et al. Interferon for treatment of breakthrough infection with hepatitis B virus mutants developing during long-term lamivudine therapy. J Gastroenterol 2002;37:922–927.
6. Lai CL, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, et al. A one-year trial of lamivudine for chronic hepatitis B. Asia hepatitis lamivudine study group. N Engl J Med 1998;339:61–68.
7. Dienstag JL, Schiff ER, Wright TL, Perrillo RP, Hann HW, Goodman Z, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med 1999;341:1256–1263.
8. Barbaro G, Zechini F, Pellicelli AM, Francavilla R, Scotto G, Bacca D, et al. Long-term efficacy of interferon alpha-2b and lamivudine in combination compared to lamivudine monotherapy in patients with chronic hepatitis B. An Italian multicenter, randomized trial. J Hepatol 2001;35:406–411.
9. Liaw YF. Management of YMDD mutations during lamivudine therapy in patients with chronic hepatitis B. J Gastroenterol Hepatol 2002;17:S333–S337.
10. Liaw YF, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, et al. Effects of extended lamivudine therapy in Asian patients with chronic hepatitis B. Asia hepatitis lamivudine study group. Gastroenterology 2000;119:172–180.
11. Leung NW, Lai CL, Chang TT, Guan R, Lee CM, Ng KY, et al. Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. Hepatology 2001;33:1527–1532.
12. Chang TT, Lai CL, Chien RN, Guan R, Lim SG, Lee CM, et al. Four years of lamivudine treatment in Chinese patients with chronic hepatitis B. J Gastroenterol Hepatol 2004;19:1276–1282.
13. de Franchis R, Hadengue A, Lau G, Lavanchy D, Lok A, McIntyre N, et al. EASL international consensus conference on hepatitis B. 13–14 September, 2002 Geneva, Switzerland. Consensus statement (long version). J Hepatol 2003;39:S3–S25.
14. Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005;365:123–129.

15. Heermann, K.H., W.H. Gerlich, M. Chudy, S. Schaefer, and R. Thomssen, Quantita tive detection of hepatitis B virus DNA in two international reference plasma preparations. EurohepPathobiology Group. J Clin Microbiol, 1999. 37(1): p. 68-73.

16. Pas, S.D., E. Fries, R.A. De Man, A.D. Osterhaus, and H.G. Niesters, Development of a quantitative real-time detection assay for hepatitis B virus DNA and comparison with two commercial assays. J Clin Microbiol, 2000. 38(8): p. 2897-901.

17. Cooksley, G., The treatment of hepatitis B e antigen-positive chronic hepatitis B with pegylated interferon. J Hepatol, 2003. 39 Suppl 1: p. S143-5.

18. Lau, G.K., T. Piratvisuth, K.X. Luo, P. Marcellin, S. Thongsawat, G. Cooksley, et al., Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med, 2005. 352(26): p. 2682-95.

19. Flink HJ, van Zonneveld M, Hansen BE, et al. Treatment with Peg-interferon alpha-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype. Am J Gastroenterol. 2006 Feb;101(2):297-303.

20. Wai, C.T., C.J. Chu, M. Hussain, and A.S. Lok, HBV genotype B is associated with better response to interferon therapy in HBeAg(+) chronic hepatitis than geno type C. Hepatology, 2002. 36(6): p. 1425-30.

21. Kao, J.H., P.J. Chen, M.Y. Lai, and D.S. Chen, Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. Gastroenterology, 2000. 118(3): p. 554-9.

22. Kao, J.H., N.H. Wu, P.J. Chen, M.Y. Lai, and D.S. Chen, Hepatitis B genotypes and the response to interferon therapy. J Hepatol, 2000. 33(6): p. 998-1002.

23. Cooksley, W.G., T. Piratvisuth, S.D. Lee, V. Mahachai, Y.C. Chao, T. Tanwandee, et al., Peginterferon alpha-2a (40 kDa): an advance in the treatment of hepatitis B e antigen-positive chronic hepatitis B. J Viral Hepat, 2003. 10(4): p. 298-305.

24. Lin, C.L., S.L. Tsai, T.H. Lee, R.N. Chien, S.K. Liao, and Y.F. Liaw, High frequency of functional anti-YMDD and -mutant cytotoxic T lymphocytes after in vitro expan sion correlates with successful response to lamivudine therapy for chronic hepa titis B. Gut, 2005. 54(1): p. 152-61.

25. Perrillo, R., H.W. Hann, D. Mutimer, B. Willems, N. Leung, W.M. Lee, et al., Adefovir dipivoxil added to ongoing lamivudine in chronic hepatitis B with YMDD mutant hepatitis B virus. Gastroenterology, 2004. 126(1): p. 81-90.

26. Levine, S., D. Hernandez, G. Yamanaka, S. Zhang, R. Rose, S. Weinheimer, et al., Efficacies of entecavir against lamivudine-resistant hepatitis B virus replication and recombinant polymerases in vitro. Antimicrob Agents Chemother, 2002. 46(8): p. 2525-32.

27. Sherman, M., C. Yurdaydin, J. Sollano, M. Silva, Z. Goodman, L. Chen, et al., Entecavir is Superior to Continued Lamivudine for the Treatment of Lamivudine-refractory, HBeAg(+) Chronic Hepatitis B: Results of Phase III Study ETV-026. Hepatology, 2004. 40(4, Suppl.1): p. 664A.

Chapter 7

Relapse after treatment with peginterferon alfa-2b alone or in combination with lamivudine in HBeAg- positive chronic hepatitis B

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ABSTRACT

Background: Interferon-induced loss of HBeAg is generally durable in chronic hepatitis B infected patients. We investigated the frequency of relapse after peginterferon alfa-2b (Peg-IFN α-2b) therapy alone or in combination with lamivudine in HBeAg positive patients.
Methods: 266 HBeAg positive patients were treated with Peg-IFN α-2b (100µg/week) with placebo (n=36) or lamivudine (100mg/day) (n=30) for 52 weeks. Post-treatment follow-up lasted 26 weeks. Relapse was defined as HBeAg negativity at week 52 and recurrence of HBeAg at week 78.
Results: HBeAg loss was observed in 44% of patients on combination therapy and 29% on monotherapy at the end of treatment ($p = 0.01$). Relapse occurred more often in patients receiving combination therapy than Peg-IFN α-2b alone (39% vs. 13%, $p = 0.005$). In the combination therapy group, relapse occurred significantly less frequent if anti-HBe was detectable at the end of treatment (21% vs. 63%, $p = 0.002$). HBV DNA below 10,000 copies/mL at the end of treatment resulted in a decreased risk of relapse ($p = 0.01$). In multivariate analysis, combination therapy (RR 3.9, CI95% 1.1-13.2, $p = 0.03$), HBsAg positivity at week 52 (RR 10.3, CI95% 1.09-97.89, $p = 0.04$) and absence of anti-HBe at week 52 (RR 9.8, CI95% 3.2-30.3, $p < 0.001$) independently predicted HBeAg relapse.
Conclusion: HBeAg relapse occurs more frequently after Peg-IFN α-2b and lamivudine combination therapy than after Peg-IFN α-2b monotherapy. Absence of anti-HBe at the end of treatment was found to be the strongest predictor of relapse. Full HBeAg seroconversion with appearance of anti-HBe, rather than HBeAg loss, thus seems the best endpoint of Peg-IFN therapy in HBeAg-positive chronic HBV.

INTRODUCTION

Sustained loss of hepatitis B e antigen (HBeAg) from serum is associated with loss of hepatitis B surface antigen (HBsAg), reduced incidence of hepatocellular carcinoma (HCC) and improved survival[1]. Treatment with interferon (IFN) for chronic hepatitis B virus (HBV) infection results in HBeAg loss in about one-third of HBeAg positive patients[2]. IFN-induced HBeAg loss is generally durable, with sustained response in up to 90% of patients [1, 3, 4]. A study by Song et al. in a Korean population demonstrated older age and presumed vertical transmission of HBV to be independent predictors for relapse after conventional IFN treatment[5].

Nucleo(t)side analogues are well capable of inhibiting HBV replication, and have been shown to induce HBeAg loss in 15-30% of patients after 1 to 2 years of treatment[6, 7]. However, prolonged treatment with lamivudine or adefovir is associated with the emergence of therapy resistant HBV strains and relapse occurs frequently after cessation of treatment with these drugs[8, 9]. Predictors for sustained HBeAg seroconversion after lamivudine therapy include prolonged duration of therapy after HBeAg seroconversion and HBV genotype[10]. Shorter duration of undetectable HBV DNA (<0.7 [10]log IU/mL) was found to predict relapse after lamivudine therapy[11]. Increased duration of undetectable HBV DNA by an additional month reduced the risk of relapse by 50%.

Recently performed studies with one year of pegylated interferon (Peg-IFN) alone or in combination with lamivudine in HBeAg positive patients showed high baseline alanine aminotransferase (ALT), low baseline HBV DNA, absence of previous IFN therapy, low baseline HBeAg and HBV genotype as independent predictors of response[12-14]. Predictors for relapse after Peg-IFN therapy are still unknown. In this study we investigated the frequency and possible predictors of relapse after treatment with Peg-IFN α-2b alone or in combination with lamivudine.

PATIENTS AND METHODS

Data for this study were extracted from a multicenter randomized controlled trial which compared Peg-IFN α-2b monotherapy to its combination with lamivudine in patients with HBeAg positive chronic hepatitis B. The inclusion and exclusion criteria were reported previously[12]. In short, patients were eligible if they had been HBsAg positive for more than 6 months, were HBeAg positive on two occasions within 8 weeks prior to randomization, had elevated serum ALT of at least twice the upper limit of normal (ULN), and had serum HBV DNA >1.0 x 10^5 copies/mL. Major exclusion criteria were: antiviral therapy within 6 months prior to randomization, serum antibodies against hepatitis C virus, hepatitis D virus

or human immunodeficiency virus (HIV), pre-existent leucopenia or thrombocytopenia (white blood cell count (WBC) ≤ 3,000/mm^3, neutrophils ≤ 1,800/mm^3, platelets ≤ 100,000/mm^3), or decompensated liver disease.

Patients were randomized in a 1:1 ratio to receive Peg-IFN α-2b (100 µg weekly) with placebo or with lamivudine (100 mg daily) for 52 weeks. After 32 weeks, Peg-IFN α-2b dosage was lowered to 50 µg to prevent side effects and early treatment discontinuation. Follow-up after discontinuation of therapy lasted 26 weeks.

During therapy and post-treatment follow-up, patients were monitored monthly by routine physical examination, as well as biochemical and hematological assessments. ALT was assessed locally and therefore expressed as times upper limits of normal (ULN). HBV DNA was assessed monthly using an in-house developed Taqman PCR assay (lower limit of detection 400 copies/mL) based on the Eurohep standard[15]. HBeAg and HBsAg (AxSYM, Abbott, Abbott Park, IL, USA) were assessed at week 0, 32, 52 and week 78 (end of follow-up). HBV genotype and YMDD mutation analysis were performed by INNO-LiPA Assay (Innogenetics, Gent, Belgium).

Liver histology was assessed at baseline in all patients. Biopsy at the end of treatment was optional. Paired biopsies were available for 110 patients. Histological scoring was performed by one experienced pathologist according to the histological activity index, modified by Ishak[16]. The pathologist was blinded for information about the chronological order of biopsies, treatment allocation and outcome measures. Improvement of histology was defined as a reduction of at least two points in necroinflammatory score (range 0-18) or one point in fibrosis score (range 0-6).

Relapse was defined as HBeAg negativity at the end of treatment (week 52) and recurrence of HBeAg at the end of follow-up (week 78). Post-treatment response was defined as HBeAg positivity at week 52 and HBeAg negativity at week 78. Virological response was defined as HBV DNA < 200,000 copies/mL and biochemical response as normalization of ALT[12]. For the assessment of virological and biochemical relapse, and post-treatment response, criteria similar to those for HBeAg response were used. The first time point of biochemical or virological response was defined as serum ALT >1 xULN or HBV DNA < 200,000 copies/mL on two consecutive occasions, respectively.

Statistical analysis was performed using the SPSS 11.5 program (SPSS Inc. Chicago, IL). Chi-square, Fisher's exact test and Mann-Whitney U test were used where appropriate. The relation between patient characteristics at baseline and during therapy, and relapse and post-treatment response was examined by logistic regression analyses. Univariate analysis was used to assess the importance of prognostic factors. To investigate the independence of these factors, multivariate logistic regression analyses was performed with all characteristics with a p-value < 0.20 in univariate analysis. A p-value < 0.05 was considered statistically significant (all two-tailed).

RESULTS

At the end of treatment, 97 of 266 patients (36%) lost HBeAg, 57 (44%) in the combination therapy group and 40 (29%) in the monotherapy group (p = 0.01). Twenty-seven of these 97 responders (28%) had recurrence of HBeAg at the end of follow-up (relapse), and 70 (72%) remained HBeAg negative throughout post-treatment follow-up (sustained response). Virological response (HBV DNA <200,000 copies/ml) occurred in 96 (74%) patients receiving combination therapy and 40 (29%) receiving monotherapy at the end of treatment (p < 0.001); ALT normalization was observed in 66 (51%) and 46 (33%) patients at the end of therapy, respectively (p = 0.005). Baseline characteristics of patients with HBeAg relapse and sustained HBeAg response are shown in Table 1. No differences in baseline variables were found between these groups.

Relapse and post-treatment response for HBeAg, ALT and HBV DNA

HBeAg relapse occurred more often in patients treated with Peg-IFN α-2b and lamivudine combination therapy compared to Peg-IFN α-2b alone (Figure 1A): 22 patients (39%) in the combination therapy group and 5 (13%) in the monotherapy group relapsed (p = 0.005). Post-treatment response occurred in 15% of patients from both treatment groups. Relapse and post-treatment response rates for biochemical and virological response are shown in Figure 1B and C. For both endpoints, patients in the combination therapy group more often relapsed than those in the monotherapy group (p = 0.054 and p = 0.037 for virological response and biochemical response, respectively). Patients with HBeAg relapse were more likely to have relapse of HBV DNA >200,000 copies/mL than sustained HBeAg responders (76% vs. 20%, p < 0.001), as well as relapse of ALT (69% vs. 24%, p = 0.007).

Prediction of HBeAg relapse

Among patients treated with combination therapy, 7 of 33 patients (21%) with detectable anti-HBe at the end of therapy relapsed compared to 15 of 24 patients (63%) without detectable anti-HBe (p = 0.002). A similar trend was observed in patients treated with Peg-IFN α-2b alone, 2 of 30 patients (7%) with detectable anti-HBe and 3 of 7 patients without (30%) relapsed (p = 0.09). Seven of 10 patients (70%) in the monotherapy group did not relapse despite the absence of anti-HBe compared to 9 of 24 patients (38%) treated with combination therapy (p = 0.13). Three patients treated with Peg-IFN α-2b alone (30%) and 4 treated with combination therapy (17%), who had no detectable anti-HBe at the end of treatment, developed anti-HBe at the end of follow-up (p = 0.39). HBV genotype tended to influence HBeAg relapse rates, 29% of patients harboring genotype A relapsed compared to 56% of patients with genotype D (p = 0.09).

Table 1. Baseline characteristics of responders at the end of treatment (n=97), divided by the occurrence of relapse (n=27) or sustained response (n=70) after therapy.

Variable	Relapse (n = 27)	Sustained response (n = 70)	p
Age*	36.3 ± 11.1	38.1 ± 12.4	0.50
Weight*	76.1 ± 14.1	75.6 ± 16.7	0.91
Race			
Caucasian	22 (82%)	52 (74%)	0.64
Asian	4 (15%)	12 (17%)	
Other	1 (3%)	6 (9%)	
Serum ALT*	5.5 ± 3.7	4.6 ± 3.0	0.22
HBV DNA (log10 copies/ml)*	9.0 ± 1.0	8.9 ± 0.9	0.66
HBV genotype			
A	11 (41%)	35 (50%)	0.22
B	2 (7%)	6 (9%)	
C	2 (7%)	8 (11%)	
D	12 (44%)	16 (23%)	
Histology †			
Necroinflammation	5 (2 – 10)	6 (2 – 10)	0.36
Fibrosis	3 (0 – 6)	3 (0 – 6)	0.69

*Mean ± SD. †Median (range).

Serum HBV DNA in relation to HBeAg relapse

Mean HBV DNA levels in patients with HBeAg relapse and sustained responders are shown in Figure 2 for patients treated with Peg-IFN α-2b alone or its combination with lamivudine. In patients treated with Peg-IFN α-2b alone, combination of HBeAg loss and HBV DNA <10,000 copies/mL at week 52 was associated with a significantly lower rate of relapse compared to HBeAg loss alone (0% vs. 63%, p = 0.01), while this difference was not observed in the combination therapy group. In none of 19 patients treated with Peg-IFN α-2b alone, who had HBV DNA <10,000 copies/mL for at least 4 weeks prior to discontinuation of therapy HBeAg relapse occurred, while this occurred in 5 of 20 patients (25%) with HBV DNA >10,000 copies/mL for at least 4 weeks relapsed (p = 0.04). At the end of follow-up, 56% of patients with sustained HBeAg response had HBV DNA <10,000 copies/mL compared to 4% of patients with relapse (p < 0.001).

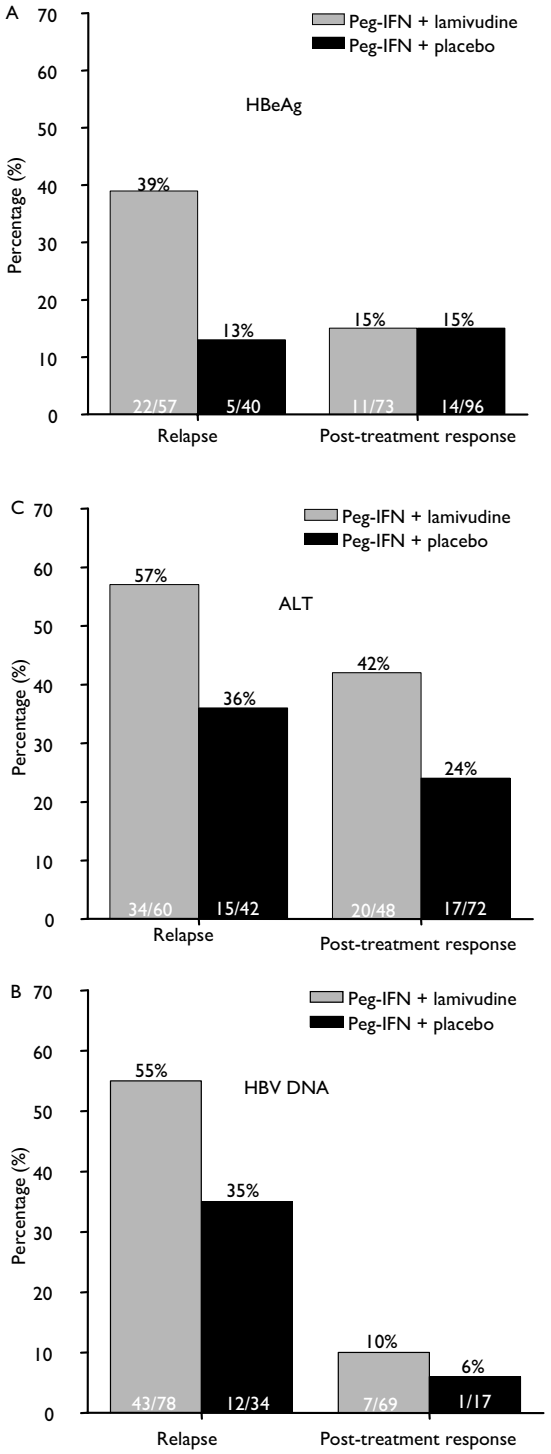

Figure 1. Relapse and post-treatment response after Peg-IFN α-2b alone or its combination with lamivudine. HBeAg relapse (HBeAg-negative at week 52 and positive at week 78) occurred significantly more often after Peg-IFN α-2b and lamivudine combination therapy compared to treatment with Peg-IFN α-2b alone (p = 0.005), as well as HBV DNA relapse (HBV DNA >200,000 copies/mL at week 78 after initial decline below this level at week 52, p= 0.054) and ALT relapse after initial normalization (p = 0.037). Post-treatment response for HBeAg and HBV DNA <200,000 copies/ml was observed equally among treatment groups, while normalization of ALT occurred more often in patients treated with Peg-IFN α-2b alone (p = 0.036).

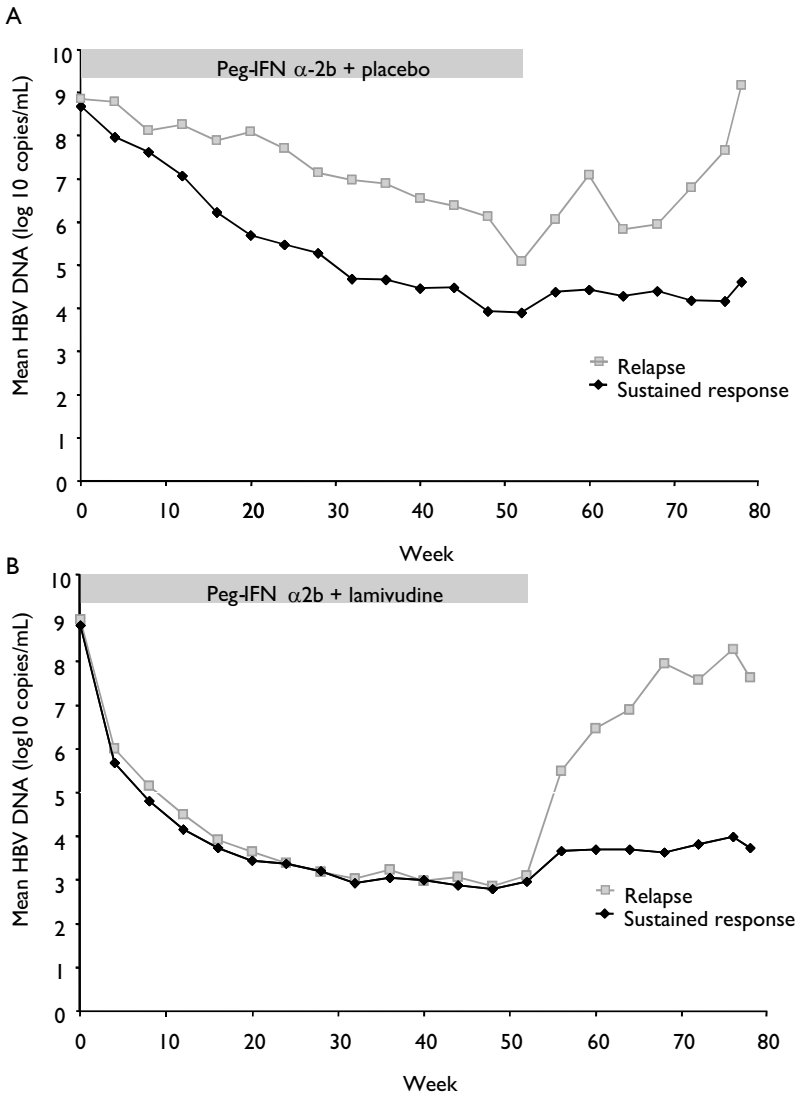

Figure 2. Mean HBV DNA in patients with HBeAg relapse and sustained response after Peg-IFN α-2b alone or its combination with lamivudine. Among patients treated with Peg-IFN α-2b monotherapy, decline in HBV DNA was more pronounced in sustained responders than relapsers (A). This difference was however not observed in patients treated with Peg-IFN α-2b and lamivudine combination therapy (B). Mean HBV DNA was comparable in sustained responders and relapsers at baseline in both treatment groups. In patients treated with Peg-IFN α-2b alone, decline in HBV DNA was 4.8 $\log^{10}$ copies/mL for patients with relapse compared to 3.8 $\log^{10}$ copies/mL for sustained responders at the end of treatment ($p = 0.30$). Decline in HBV DNA was 5.8 $\log^{10}$ copies/mL for all patients in the combination therapy group at the end of treatment. In both treatment groups mean HBV DNA was significantly higher in relapsers than sustained responders at week 78 (9.2$\log^{10}$ vs. 4.6 $\log^{10}$ for monotherapy and 7.7 $\log^{10}$ vs. 3.7 $\log^{10}$ for combination therapy, $p < 0.001$).

Logistic regression analysis of factors predicting HBeAg relapse and post-treatment HBeAg response

For the prediction of HBeAg relapse and post-treatment HBeAg response, the following baseline variables were included in univariate logistic regression analysis: age, sex, weight, race, mode of transmission, serum ALT, HBV DNA level, HBV genotype and liver histology. In addition to these baseline variables, treatment allocation, HBeAg and anti-HBe status at week 32; serum ALT, HBV DNA, anti-HBe, HBsAg (HBeAg relapse only) and liver histology at week 52; and time points of ALT normalization and HBV DNA response were included (Table 2). In multivariate time dependent analysis, combination therapy of Peg-IFN α-2b with lamivudine (RR 3.9, CI95% 1.1 - 13.2, p = 0.03), HBsAg negativity at week 52 (RR 10.3, CI95% 1.09-97.89, p = 0.04) and absence of anti-HBe at week 52 (RR 9.8, CI95% 3.2 - 30.3, p < 0.001) independently predicted HBeAg relapse. Absence of anti-HBe at the end of treatment was found to be the strongest predictor of HBeAg relapse. Weight was found to be the only independent predictor of post-treatment HBeAg response, higher weight decreased the likelihood of HBeAg response after discontinuation of therapy (RR 0.92, 95% CI 0.87-0.97, p = 0.003).

Table 2. Factors significantly associated with HBeAg relapse and post-treatment response in univariate analysis.

Variable	Hazard ratio	95% CI		p
		lower	upper	
Relapse				
Combination therapy	4.4	1.15	12.93	0.007
Anti-HBe positive week 32	0.29	0.10	0.82	0.02
Anti-HBe positive week 52	0.15	0.06	0.39	<0.001
Post-treatment response				
Sex – male	0.25	0.11	0.61	0.002
Weight	0.95	0.92	0.99	0.01
Body mass index (BMI)	0.90	0.82	0.99	0.03
Baseline necroinflammation	1.25	1.00	1.55	0.05
HBeAg negative week 32	13.16	3.03	57.09	0.001
Anti-HBe positive week 32	8.03	1.98	32.58	0.004
Time point of ALT normalization	0.98	0.96	0.99	0.007
Time point of HBV DNA response	0.98	0.97	1.00	0.009

DISCUSSION

In HBeAg-positive chronic hepatitis B, relapse occurs often after discontinuation of nucleos(t)ide analogue therapy, while response appears more durable after interferon-based treatment because of its immunomodulatory effects. In the current study we found 39% relapse after discontinuation of Peg-IFN α-2b and lamivudine combination therapy and 13% after Peg-IFN α-2b alone. Observed relapse rates are consistent with findings of previous studies, which showed HBeAg relapse in 22-40% of lamivudine treated patients[3,17], and 10% of IFN treated patients[3,18]. Most likely, the higher relapse rates (preceded by higher response rates) after cessation of combination therapy compared to Peg-IFN α-2b monotherapy can be explained by lamivudine induced HBeAg loss.

In multivariate analysis, absence of anti-HBe and HBsAg-negativity at the end of treatment independently predicted HBeAg recurrence in addition to treatment allocation. Factors predicting relapse after conventional IFN have previously been described and include older age and presumable vertical transmission[5]. We did not find a relation between relapse and these factors in both univariate and multivariate analysis. Possibly our findings differ with findings from this study because of differences in patient population (Asian vs. Caucasian population) and type or duration of the antiviral therapy.

Among Peg-IFN α-2b and lamivudine treated patients in our study, 21% of those positive for anti-HBe at the end of therapy relapsed compared to 63% of patients who lacked anti-HBe. In HBeAg-positive patients treated with Peg-IFN α-2a, HBeAg seroreversion occurred in 18% of patients treated with Peg-IFN α-2a monotherapy and 22% of patients on combination therapy with lamivudine[19]. Response was durable in 86% of responders at 1 year post-treatment in this study[20]. Although relapse rates in patients without detectable anti-HBe in our study are much higher compared to Peg-IFN α-2a treated patients with HBeAg-seroconversion, relapse rates for patients with loss of HBeAg and detectable anti-HBe at the end of treatment seem comparable between the two studies.

Presence of anti-HBe may be less important for Peg-IFN α-2b monotherapy than combination therapy, since the majority of patients treated with Peg-IFN α-2b monotherapy (7 of 10 patients) did not relapse despite the absence of anti-HBe at the end of therapy. In patients with HBeAg relapse, HBV DNA levels declined during treatment, but decline was less profound compared to sustained responders (Figure 2). HBV DNA <10,000 copies/mL prior to discontinuation of treatment was found to significantly decrease the risk of relapse in Peg-IFN α-2b treated patients. This is consistent with findings on relapse after cessation of lamivudine in patients with prolonged suppression of HBV DNA[11]. Decline in HBV DNA (and ALT normalization) may thus be important for maintaining Peg-IFN induced response.

Patients with a partial response as defined by HBeAg loss but insufficient decline of HBV DNA (not below 10,000 copies/mL) at the end of therapy, might benefit from prolonged therapy, as has previously been shown to increase response to conventional IFN[21].

Maintaining HBV DNA below 10,000 copies/mL after discontinuation of therapy seems important since recent studies showed that serum HBV DNA below this level reduces the risk of progression to cirrhosis, decompensated liver disease and HCC[22, 23]. Fifty-six percent of sustained responders had HBV DNA <10,000 copies/mL at the end of follow-up, while virtually all patients with relapse had HBV DNA above this level. In HBeAg positive patients treated with monotherapy of Peg-IFN α-2a, a comparable rate of HBV DNA below 10,000 copies/mL was observed in responders (66%) at the end of follow-up[24].

HBV genotype independently predicted loss of both HBeAg and HBsAg in HBeAg-positive patients treated with Peg-IFN α-2b, with higher response rates in patients with genotype A compared to those with genotype D[12, 25]. A similar trend was observed in a population of mainly Asian patients treated with Peg-IFN α-2a[19, 26]. HBV genotype also tended to influence HBeAg relapse after discontinuation of Peg-IFN α-2b with or without lamivudine, with genotype D infected patients having a higher risk of relapse than those with genotype A.

In conclusion, HBeAg relapse occurs more often after treatment of Peg-IFN α-2b in combination with lamivudine than after Peg-IFN α-2b monotherapy. Absence of anti-HBe at the end of treatment was found to be the strongest predictor of relapse, particularly in patients treated with combination therapy. HBeAg loss thus is less suitable as sole endpoint of Peg-IFN based therapy in HBeAg positive chronic HBV. More profound suppression of HBV DNA (below 10,000 copies/mL) decreases the risk of relapse. Patients with a partial response might benefit from prolonged Peg-IFN treatment, further reducing HBV DNA and thereby possibly increasing the development of anti-HBe and reducing the risk of relapse after discontinuation of therapy.

REFERENCES

1. van Zonneveld M, Honkoop P, Hansen BE, Niesters HGM, Darwish Murad S, de Man RA, et al. Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. Hepatology. 2004;39(3):804-10.
2. Wong DK, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. Ann Intern Med. 1993 Aug 15;119(4):312-23.
3. van Nunen AB, Hansen BE, Suh DJ, Lohr HF, Chemello L, Fontaine H, et al. Durabil ity of HBeAg seroconversion following antiviral therapy for chronic hepatitis B: relation to type of therapy and pretreatment serum hepatitis B virus DNA and alanine aminotransferase. Gut. 2003 Mar;52(3):420-4.
4. Korenman J, Baker B, Waggoner J, Everhart JE, Di Bisceglie AM, Hoofnagle JH. Long-term remission of chronic hepatitis B after alpha-interferon therapy. Ann Intern Med. 1991 Apr 15;114(8):629-34.
5. Song BC, Suh DJ, Lee HC, Chung YH, Lee YS. Which patients with chronic hepatitis B are more likely to relapse after interferon alpha-induced hepatitis B e antigen loss in Korea? J Clin Gastroenterol. 2004 Feb;38(2):124-9.
6. Marcellin P, Chang TT, Lim SG, Tong MJ, Sievert W, Shiffman ML, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N Engl J Med. 2003 Feb 27;348(9):808-16.
7. Dienstag JL, Schiff ER, Wright TL, Perrillo RP, Hann HW, Goodman Z, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med. 1999 Oct 21;341(17):1256-63.
8. Leung NW, Lai CL, Chang TT, Guan R, Lee CM, Ng KY, et al. Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. Hepatology. 2001 Jun;33(6):1527-32.
9. Sung JJ, Wong ML, Bowden S, Liew CT, Hui AY, Wong VW, et al. Intrahepatic hepa titis B virus covalently closed circular DNA can be a predictor of sustained responseto therapy. Gastroenterology. 2005 Jun;128(7):1890-7.
10. Chien RN, Yeh CT, Tsai SL, Chu CM, Liaw YF. Determinants for sustained HBeAg response to lamivudine therapy. Hepatology. 2003 Nov;38(5):1267-73.
11. Ito K, Tanaka Y, Orito E, Hirashima N, Ide T, Hino T, et al. Predicting relapse after cessation of Lamivudine monotherapy for chronic hepatitis B virus infection. Clin Infect Dis. 2004 Feb 15;38(4):490-5.
12. Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial.
Lancet. 2005 Jan 8;365(9454):123-9.

13. Cooksley G, Lau GKK, Liaw YF, Marcellin P, Chow WC, Thongsawat S, et al. Effects of genotype and other baseline factors on response to peginterferon alfa-2a (40 kDa) (Pegasys®) in HBeAg-positive chronic hepatitis B: results from a large, randomised study. J Hepatol. 2005;42(Suppl.2):S30.

14. Bonino F, Lau GKK, Marcellin P, Hadziyannis S, Kitis G, Jin R, et al. The first detailed analysis of predictors of response in HBeAg-negative chronic hepatitis B: data from a multicenter, randomized, partially double-blind study of peginterferon-alfa-2a (40-KD) (Pegasys®) alone or in combination with lamivudine vs lamivudine alone. Hepatology. 2004;40(S4):A1142.

15. Pas SD, Fries E, De Man RA, Osterhaus AD, Niesters HG. Development of a quan titative real-time detection assay for hepatitis B virus DNA and comparison with two commercial assays. J Clin Microbiol. 2000;38(8):2897-901.

16. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. J Hepatol. 1995 Jun;22(6):696-9.

17. Yoon SK, Jang JW, Kim CW, Bae SH, Choi JY, Choi SW, et al. Long-term results of lamivudine monotherapy in Korean patients with HBeAg-positive chronic hepati tis B: response and relapse rates, and factors related to durability of HBeAg seroconversion. Intervirology. 2005 Nov-Dec;48(6):341-9.

18. van Zonneveld M, Honkoop P, Hansen BE, Niesters HG, Murad SD, de Man RA, et al. Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. Hepatology. 2004 Mar;39(3):804-10.

19. Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, et al. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med. 2005 Jun 30;352(26):2682-95.

20. Lau DT, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, et al. Durabil ity of response and occurrence of late response to peginterferon alpha-2a (40KD) one year post-treatment in patients with HBeAg-positive chronic hepatitis B. J Hepatol. 2006;44(Suppl. 2):S23.

21. Janssen HL, Berk L, Schalm SW, Heijtink RA, Hess G, Rossol S, et al. Antiviral effect of prolonged intermittent lymphoblastoid alpha interferon treatment in chronic hepatitis B. Gut. 1992 Aug;33(8):1094-8.

22. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. 2006 Jan 4;295(1):65-73.

23. Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology. 2006 Mar;130(3):678-86.

24. Piratvisuth T, Lau GKK, Marcellin P, Chow WC, Cooksley G, Fried MW, et al. Asso ciation between HBeAg seroconversion and sustained HBV DNA suppression in patients treated with peginterferon alfa-2a (40KD) for HBeAg-positive chronic hepatitis B. J Hepatol. 2006;44(Suppl.2):S23.

25. Flink HJ, van Zonneveld M, Hansen BE, de Man RA, Schalm SW, Janssen HL. Treatment with peg-interferon alpha-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype. Am J Gastroenterol. 2006 Feb;101(2):297-303.

26. Hadziyannis S, Lau GKK, Marcellin P, Piratvisuth T, Cooksley G, Bonino F, et al. Sustained HBsAg seroconversion in patients with chronic hepatitis B treated with peginterferon alpha-2a (40kDa) (Pegasys®). J Hepatol. 2005;42(Suppl 2):S178.

Chapter 8

Is weight-based dosing treatment necessary for peginterferon alfa-2b therapy in HBeAg-positive chronic hepatitis B?

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ABSTRACT

Weight-based dosing of peginterferon alfa-2b (Peg-IFN α-2b) therapy is probably more effective than a fixed dose for the treatment of chronic hepatitis C. In this study we investigated whether body weight was associated with response (HBeAg loss at end of follow-up) after Peg-IFN α-2b therapy. A total of 266 chronic HBeAg-positive hepatitis B patients received 100 µg Peg-IFN α-2b weekly for 52 weeks. After week 32 the dose was halved to 50 µg Peg-IFN α-2b. The post-treatment follow-up lasted 26 weeks. Using this fixed dose of Peg-IFN α–2b HBeAg clearance decreased with higher bodyweight until 85 kg: HBeAg response was 46% for ≤ 55 kg (n=26), 42% for 55-65 kg (n=65), 31% for 65-75 kg (n=77), 24% for 75-85 kg (n=58), and 45% for > 85 kg (n=44). Multivariate analysis did not show interaction between patients > 85 kg and baseline ALT, HBV DNA or previous treatment with interferon. Among patients > 85 kg, 41% (n=18) harbored genotype A, and 47% (n=21) genotype D. Interestingly, within genotype A, patients > 85 kg responded better than patients ?85 kg, (83% vs. 39%, p = 0.001), the opposite was found for genotype D, 8% vs. 29% for patients > 85kg and ≤ 85 kg, respectively, p = 0.06). Body mass index (BMI) was not significantly different between genotype A (mean BMI = 24) and genotype D (mean BMI = 24), p = 0.92.

In conclusion, our study indicates that Peg-IFN α-2b should probably be administered on weight-based dosing regimen for patients harboring genotype D. Weight-based dosing may not be necessary for patients harboring genotype A. Dose and duration ranging studies are needed for further optimalization of Peg-IFN α-2b therapy.

INTRODUCTION

World-wide, approximately 400 million people are chronically infected with the hepatitis B virus (HBV), and chronic hepatitis B (CHB) infection has become the single most common cause for decompensated liver disease and hepatocellular carcinoma[1]. CHB can be treated with nucleos(t)ide analogues such as lamivudine and adefovir or with (pegylated) interferon. Pegylated interferons (Peg-IFNs) are already widely used in the treatment for chronic hepatitis C but have only recently been introduced for the treatment of CHB[2-4]. For the treatment of hepatitis C two types of Peg-IFNs have been registered. Peginterferon alfa-2a (Peg-IFN α-2a) which is administered using a fixed-dose regimen, and peginterferon alfa-2b (Peg-IFN α-2b) which is weight-based administered. In hepatitis C studies with a fixed-dose of Peg-IFN α-2a, increased body weight led to a lower rate of sustained virological response[5, 6]. Another study by Lindsay et al. in which Peg-IFN α-2b was used on a weight-based dosing regimen, did not show that body weight was a predictive factor for response[7].

As yet, it is unknown whether body weight is associated with response in patients infected with CHB. In a recently conducted study using a fixed-dose of Peg-IFN α-2b for HBeAg-positive chronic hepatitis B[4], we investigated whether body weight was associated with HBeAg loss at the end of follow-up after treatment with Peg-IFN α-2b with or without lamivudine.

PATIENTS AND METHODS

For this study, we extracted the data from a multicenter randomized controlled trial which compared Peg-IFN α-2b mono-therapy to Peg-IFN α-2b combination therapy with lamivudine in chronic hepatitis B[4]. The in- and exclusion criteria were reported previously. Major inclusion criteria were: HBeAg-positive on two occasion[8] weeks prior to randomization, elevated serum alanine transferase (ALT) of at least twice the upper limit of normal (ULN), and serum HBV DNA $>10^5$ copies/ml. Patients were assigned to 100 µg Peg-IFN α-2b with placebo or with daily 100 mg lamivudine for 52 weeks. After 32 weeks the dose of Peg-IFN α-2b was halved into 50µg in order to prevent side effects and early treatment discontinuation. Patients who weighted $\leq$ 55kg were treated according to a weight-based regimen (1.5 µg/kg for the first 32 weeks and 0.75 µg/kg for the remaining treatment period). Dose of Peg-IFN α-2b for hematological abnormalities was reduced with 50% in the event of neutropenia (granulocytes $< 1.0 \times 10^9$/L), leucopenia (white blood cells $< 2.0 \times 10^9$/L), and thrombocytopenia (platelets $< 50 \times 10^9$/L). Other clinical relevant adverse events associated with the use of Peg-IFN α-2b (such as depression, fatigue and flu-like-symptoms) were also reasons for dose reduction. Post-treatment follow-up lasted 26 weeks. During therapy and follow-up patients were seen every 4 weeks for routine examination and biochemical and

hematological assessments. Transaminases were determined locally and therefore expressed as times upper limits of normal (ULN). HBV DNA was also determined 4-weekly using an in-house Taqman PCR (detection limit 400 copies/mL) based on the Eurohep standard[8]. HBeAg (AxSYM, Abbott) and HBsAg (AxSYM, Abbott) status was determined at weeks 0, 32, 52 (during treatment) and at week 78 (end of follow-up). HBV genotype was assessed by Inno-Lipa Assay, Innogenetics.

Liver histology was assessed at baseline and an optional biopsy was performed at the end of treatment. Paired biopsies were available for 110 patients. Histological scoring was performed by one experienced pathologist according to the histological activity index, modified by Ishak[14]. The pathologist was blinded for chronological order of biopsies and treatment schedule. Improvement of histology was defined as a reduction of at least two points in the necroinflammatory score (range 0-18) and one point of the fibrosis score (range 0-6). Response was defined as HBeAg loss at the end of follow-up. ALT normalization and HBV DNA response was defined as serum ALT <1x ULN, and serum viral load <200.000 copies/mL, respectively.

Statistical analysis

All data were analyzed using SPSS version 11.5 (SPSS Inc. Chicago, IL). Chi-square, Fisher's exact test, and Mann-Whitney U test were used where appropriate. A p value < 0.05 was considered significant (all two-tailed).

RESULTS

Of the original 266 patients, 264 were categorized in 5 weight categories, ≤ 55 kg (n=26), 55-65 kg (n=59), 65-75 kg (n=77), 75-85 kg (n=58) or >85 kg (n=44). For 2 patients data on body weight were missing. Mean total dose of Peg-IFN α-2b was 62μg/kg, 58μg/kg, 55μg/kg, 49μg/kg and 42μg/kg for the different weight groups, respectively. HBeAg clearance was seen for 46% (n=12) of patients ≤ 55 kg, 42% (n=25) of patients 55-65 kg, 31% (n=24) of patients 65-75 kg, 24% (n=14) of patients 75-85 kg, and 45% (n=20) of patients >85 kg (Figure 1). Except for patients > 85 kg HBeAg clearance decreased with increasing body weight. When excluding patients > 85 kg body weight was significantly associated with HBeAg clearance (RR 0.97 95%CI 0.95 to 1.00, p = 0.047). The addition of lamivudine did not change the response pattern nor HBeAg loss at the end of follow-up. Interestingly, patients >85 kg responded significantly better than those weighing 75-85 kg (RR 2.6 95%CI 1.1 to 6.1, p = 0.026). A similar trend was found when compared with patients weighing 65-75kg (RR 1.8 95%CI 0.9 to 4.0, p = 0.12).

Table 1. Baseline characteristics according to weight group

Baseline	≤55 kg (n = 26)	55-65 kg (n = 59)	65-75 kg (n = 77)	75-85 kg (n = 58)	> 85 kg (n = 44)
Age*	29 ± 9.9	32 ± 12.7	35 ± 12.6	36 ± 13.5	41 ± 12.4
Sex – male	4 (15%)	38 (64%)	65 (84%)	54 (93%)	42 (96%)
Race					
Caucasian	13 (50%)	39 (66%)	54 (70%)	48 (83%)	40 (91%)
Asian	12 (46%)	14 (24%)	17 (22%)	8 (14%)	1 (2%)
Other	1 (4%)	6 (10%)	6 (8%)	2 (3%)	3 (7%)
HBV Genotype					
A	4 (15%)	12 (20%)	29 (38%)	25 (43%)	18 (41%)
B	5 (19%)	8 (14%)	7 (9%)	3 (5%)	0
C	6 (23%)	11 (19%)	13 (17%)	7 (12%)	2 (5%)
D	11 (42%)	25 (42%)	24 (31%)	22 (38%)	21 (48%)
other	0	3 (5%)	4 (5%)	1 (2%)	3 (7%)
ALT (x ULN)*	3.8 ± 2.8	4.3 ± 4.5	4.1 ± 2.8	4.3 ± 2.7	5.2 ± 3.8
Log HBV DNA*	9.0 ± 1.0	8.7 ± 1.3	9.0 ± 0.9	9.4 ± 0.8	9.3 ± 0.7
Cirrhosis	0	3 (5%)	6 (8%)	7 (12%)	8 (18%)
Previous IFN	2 (8%)	14 (24%)	15 (20%)	15 (26%)	12 (27%)

* Mean ± SD

In our previous study with the same population[4], we found that higher baseline serum ALT levels, lower baseline HBV DNA levels, absence of previous IFN treatment and HBV genotype A were positive predictors for HBeAg loss at the end of follow up. To reveal whether these baseline variables could explain the marked increase of HBeAg loss at the end of follow-up of the patients > 85 kg, we performed a univariate analysis between the weight categories (Table 1). The HBV genotypes were not significantly different between the weight categories. Logistic multivariate analysis did not show any interaction between the known predictors for response or other baseline variables and patients > 85 kg.

Among patients > 85 kg 41% harbored HBV genotype A (n=8) and 47% D (n=21). As shown in Table 1, these most common HBV genotypes were not more prevalent among patients > 85 kg than other weight categories. Interestingly, within genotype A, patients > 85 kg responded better than patients ≤ 85 kg, (83% vs. 39%, p = 0.001), whereas the opposite was found for genotype D, 8% vs. 29% for patients >85kg and HBeAg loss ≤ 85 kg, respectively, p = 0.06). Figure 2 shows the relation of body weight and response according to the HBV genotype A and D. Body mass index (BMI) was not different between genotype A (mean BMI = 24) and genotype D (mean BMI = 24), p = 0.92. For BMI in relation to HBeAg loss a similar pattern was found for genotypes A and D.

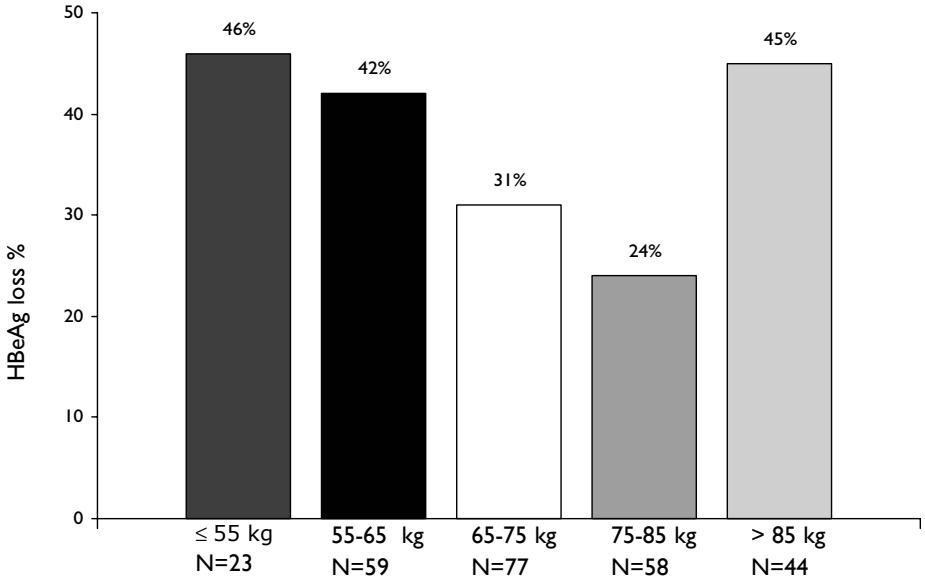

Figure 1. HBeAg loss at the end of follow-up (week 78) according to weight category. HBeAg loss for patients > 85 kg (45%) versus 75-85 kg (24%), p = 0.024.

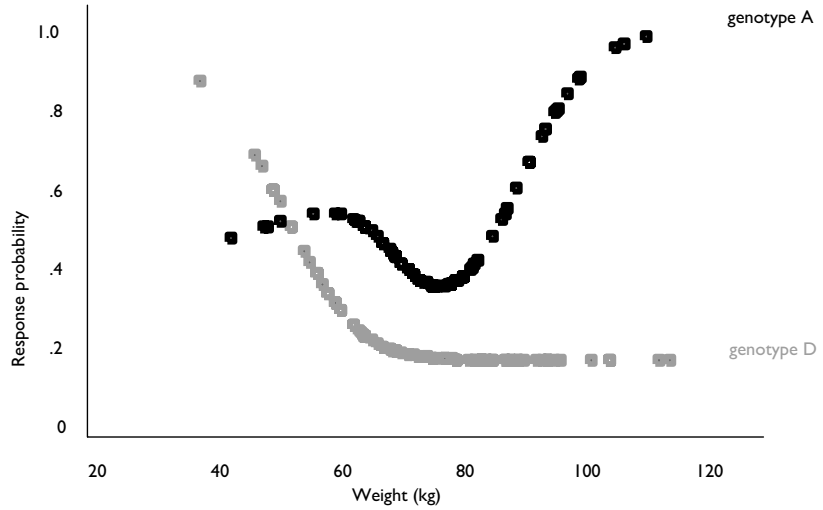

Figure 2. Body weight and relation to response probability (HBeAg loss at the end of follow-up). Among patients with HBV genotype A (black) probability of response increases in patients > 85 kg. Among patients harboring genotype D (grey) response decrease with increasing body weight.

DISCUSSION

In contrast with the treatment for chronic hepatitis C in which Peg-IFNs are widely used, Peg-IFNs for CHB therapy have only been introduced recently[2-4, 9]. In the treatment for chronic hepatitis C it is assumed that with a fixed dose of Peg-IFN α-2a higher body weight-based is associated with a lower sustained virological response[5, 6]. The role of body weight in relation to response in chronic hepatitis B has not been investigated yet. In the current study with fixed dose of Peg-IFN α-2b with or without the addition of lamivudine we found HBeAg loss for 46% of patients ≤ 55 kg, 42% of patients 55-65 kg, 31% of patients 65-75 kg, 24% of patients 75-85 kg, and 45% of patients >85 kg. Except for patients > 85 kg, patients tended to have less HBeAg loss at the end of follow-up with increasing body weight. An interesting finding of the current study was the marked increase in HBeAg loss in patients > 85 kg. One would expect that with a fixed dose of Peg-IFN α-2b higher body weight would lead to lower HBeAg loss at the end of follow-up. In previous studies with a fixed dose of Peg-IFN α-2a (with or without daily ribavirin) for chronic hepatitis C infection, they found that patients < 85 kg responded better than those ≥ 85 kg[5]. Fried et al found that patients ≤75 kg more often had sustained responses than those >75 kg [6]. Obviously, when these results are extrapolated to chronic HBV infection, the comparison of the data should be made with caution.

Possible confounders (such as high baseline ALT, low baseline HBV DNA, previous IFN and HBV genotype[4]), which could explain the marked increase of HBeAg loss in patients > 85 kg could not be identified. Although baseline serum ALT was higher among patients > 85 kg, the difference was not significant. Also genotype A and D were equally distributed within patients >85 kg, which rules out the opportunity of over representation of genotype A. Within these genotypes a remarkable finding was the fact that within genotype A HBeAg loss at the end of follow-up was very high among patients > 85 kg. Eighty-three percent of these patients showed HBeAg loss at the end of follow-up. Moreover, the opposite was found for patients harboring genotype D and > 85 kg. To our knowledge no such data has been presented on response and weight in relation to HBV genotype. The two large studies with Peg-IFN α-2a in HBeAg-positive and HBeAg-negative CHB did not report in this topic either[2, 3].

In conclusion, our study indicates that Peg-IFN α-2b should possibly be administered on weight-based dosing regimen for HBeAg-positive CHB, especially those harboring genotype D. Weight-based dosing may not be necessary for patients harboring genotype A since highest response rates are found among those > 85kg. However, these findings need to be confirmed in a larger study containing Peg-IFN α-2b therapy on a fixed dose and on a weight-based dose regimen for further optimalization of Peg-IFN α-2b therapy.

REFERENCES

1. Lee WM. Hepatitis B virus infection. N Engl J Med 1997;337(24):1733-45.
2. Lau GK, Piratvisuth T, Luo KX, et al. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005;352(26):2682-95.
3. Marcellin P, Lau GK, Bonino F, et al. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2004;351(12):1206-17.
4. Janssen HL, van Zonneveld M, Senturk H, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005;365(9454):123-9.
5. Lee SS, Heathcote EJ, Reddy KR, et al. Prognostic factors and early predictability of sustained viral response with peginterferon alfa-2a (40KD). J Hepatol 2002;37(4):500-6.
6. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347(13):975-82.
7. Lindsay KL, Trepo C, Heintges T, et al. A randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. Hepatology 2001;34(2):395-403.
8. Pas SD, Fries E, De Man RA, et al. Development of a quantitative real-time detection assay for hepatitis B virus DNA and comparison with two commercial assays. J Clin Microbiol 2000;38(8):2897-901.
9. Cooksley WG, Piratvisuth T, Lee SD, et al. Peginterferon alpha-2a (40 kDa): an advance in the treatment of hepatitis B e antigen-positive chronic hepatitis B. J Viral Hepat 2003;10(4):298-305.
10. Zeuzem S, Feinman SV, Rasenack J, et al. Peginterferon alfa-2a in patients with chronic hepatitis C. N Engl J Med 2000;343(23):1666-72.

Chapter 9

Summary and conclusions

Samenvatting en conclusies

Appendix

Dankwoord

Curriculum vitae

Bibliography

Abbreviations

SUMMARY

Word-wide, infection with the hepatitis B virus (HBV) is a major health problem. Over 2 billion people have been exposed to HBV, and approximately 400 million are chronic carriers of the virus. Chronic hepatitis B (CHB) infection may lead in term to liver cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC), and is responsible for an estimated 1 million deaths annually.

The efficacy of the established treatment for CHB is limited. Treatment with conventional interferon (IFN) is only effective in 20-35%. Lamivudine needs to be given long-term to maintain response and leads to emergence of viral resistant strains. Several studies have investigated different treatment options such as peginterferon, adefovir and entecavir. The first randomized controlled trial in HBeAg-positive CHB with peginterferon - which had proven to be more effective than conventional IFN in chronic hepatitis C patients - demonstrated that a 52-week course of peginterferon alfa-2b leads to HBeAg seroconversion in 36%, ALT normalization in 35% and HBV DNA < 2.0×10^5 copies/mL in 32% of patients at the end of post treatment follow-up. The addition of lamivudine did not enhance response rates compared to peginterferon alfa-2b alone. The use of conventional IFN is not suited for all patients due to the significant side effects and early treatment discontinuation that may occur. Although the safety profile of peginterferon has been studied in chronic hepatitis C, the side effects of peginterferon in CHB patients have not previously been investigated. We assessed the safety of peginterferon treatment in 266 HBeAg-positive patients who received peginterferon alfa-2b alone or in combination with lamivudine (chapter 2). The most common side effects were similar to those reported for conventional IFN. Flu-like symptoms, headache, fatigue, myalgia, local reaction at the injection, abdominal discomfort and psychiatric symptoms were most frequently reported, but in general subsided during the course of therapy. Hematologic abnormalities (leucopenia, neutropenia and thrombocytopenia) were frequent but did not lead to serious infections or bleeding complications. Neutropenia was the major cause for dose reductions of peginterferon, whereas psychiatric symptoms (depression, psychosis) were the most important reasons for early treatment discontinuation. Pre-existing cirrhosis at baseline was an important risk factor for thrombocytopenia and (minor) bleeding complications.

During conventional IFN therapy and in the natural history of the disease, acute exacerbations of CHB are a well-known phenomenon. Flares have been attributed to increased T cell cytolytic activity and natural killer cell function and may herald HBeAg seroconversion. Flares during treatment and follow-up of peginterferon alone or combined with lamivudine occurred in 25% of patients (chapter 3). The frequency of all flares was comparable among the HBV genotypes, but flares on-treatment were more frequent within genotype A than genotype D. Flares during treatment were associated with response to therapy in contrast

to those during post treatment follow-up. Close monitoring revealed two different types of flares; host-induced flares which were followed by a significant decrease in serum HBV DNA levels, and virus-induced flares which were preceded by an increase in serum HBV DNA. Virus-induced flares were detrimental for treatment outcome and occurred frequently after treatment. These flares possibly reflect the exacerbations of HBV as described after cessation of lamivudine and may be severe and life-threatening. In contrast, host-induced flares were the strongest predictors for HBeAg loss, 58% of patients exhibiting a host-induced flares had lost HBeAg at the end of follow-up. This further underlines that full control and elimination of the virus is achieved by a vigorous host immune response rather than by direct suppression of the virus.

In chapter 4 we investigated the frequency of HBsAg loss and the role of HBV genotype after treatment. HBsAg seroclearance or HBsAg seroconversion is being regarded as the ideal endpoint of therapy, since it is strongly associated with improved survival and reduction of HCC. Spontaneous clearance is rare and treatment with nucleoside analogues seldom leads to HBsAg seroconversion. After peginterferon therapy a total of 18 patients (7%) were HBsAg negative at the end of follow up. The frequency of HBsAg clearance was similar between the treatment groups. Among patients being HBsAg negative at the end of follow-up all had normalized serum ALT levels and HBV DNA < 2.0 105 copies/mL. Sixty-one percent was HBV DNA negative using in-house Taqman PCR (detection limit 400 copies/mL). In addition to HBeAg loss, HBV genotype was also strongly associated with HBsAg loss. We found HBsAg seroclearance in 14% of patients harbouring genotype A patients, 9% harbouring genotype B, 3% harbouring genotype C, and 2% harbouring genotype D. Among HBeAg responders, 31%, 20%, 9% and 8% of patients lost serum HBsAg, respectively. Although studies on new treatment options for CHB showed promising results, there is a growing population of patients who previously failed to respond to the established therapies with conventional IFN or lamivudine. As the efficacy of peginterferon therapy has not been investigated in these difficult-to-treat population, we studied 76 patients who previously where non-responder to IFN and or lamivudine therapy (chapter 5). Treatment of peginterferon alone or in combination with lamivudine lead to HBeAg loss in 35% of previous non-responders to conventional IFN, 29% of non-responders to lamivudine and 18% of non-responders to both therapies. ALT normalization was found in 24%, 41% and 14% of previous non-responders, and HBV DNA levels < 400 copies/mL at the end of therapy was found in 5%, 6%, and 0% of previous non-responders, respectively. Especially patients with baseline ALT levels > 4 x upper limits of normal benefit from peginterferon therapy. Previous dose and treatment duration were not associated with HBeAg loss. We performed additional analyses in patients harbouring a YMDD mutation (chapter 6). This study revealed only minor efficacy of peginterferon in patients carrying a mutation in the YMDD motif of the

HBV. Only 2 patients (13%) converted to HBeAg negativity with ALT normalization and HBV DNA levels < 2.0 x 10^5 copies/mL. This study suggests that the efficacy of peginterferon in this specific population is limited, but larger studies are needed to draw definite conclusions. Peginterferon in previous non-responders without the YMDD mutation seems beneficial. In general, the response to IFN or peginterferon is durable in CHB patients, but relapse after lamivudine therapy is frequently seen. The effect of peginterferon monotherapy or its combination with lamivudine on relapse is still unknown (chapter 7). Relapse was more often seen after cessation of peginterferon combined with lamivudine (39%) than after peginterferon alone (13%). Multivariate analysis confirmed combination therapy as independently predictive for relapse. Absence of anti-HBe at week 52 was an independent predictor. Thus, HBeAg loss seems less suitable as sole endpoint, especially for those receiving combination therapy. The development of anti-HBe in peginterferon monotherapy may be less important, since the majority of patients treated in the monotherapy (7 of 10 patients) did not relapse despite the absence of anti-HBe at the end of therapy. Further suppression of HBV DNA levels could enhance sustained response, as mean HBV DNA levels were lower in sustained responders compared to relapsers.

Increased body weight is a risk factor for non-response and relapse on peginterferon in chronic hepatitis C. Studies with peginterferon alfa-2b which is normally administered on a weight-based doses showed no relationship between increased weight and lower response rates, while studies with peginterferon alfa-2a which is based on a fixed dose regimen demonstrated decreased efficacy with higher body weight. We investigated the effect of peginterferon alfa-2b in CHB using a fixed dose of 100 µg (chapter 8). For this study, 264 patients were categorized in 5 weight categories, ≤ 55 kg (n = 26), 55-65 kg (n = 59), 65-75 kg (n = 77), 75-85 kg (n = 58) or > 85 kg (n = 44). HBeAg response for the corresponding weight groups was 46%, 42%, 31%, 24%, and 45% respectively. The vast majority of patients > 85 kg consisted of patients with genotype A or D. Within genotype A, patients >85 kg responded better than patients ≤ 85 kg, (83% vs. 39%), while the opposite was found for genotype D, 8% vs. 29% for patients > 85kg and ≤ 85 kg. In contrast to genotype D patients,

weight based dosing may not be needed in patients harbouring genotype A, but future studies are needed to optimize treatment regimens for CHB.

CONCLUSIONS

1. Peginterferon alfa-2b is safe in HBeAg-positive chronic hepatitis B. Most side effects occur during the first months of treatment, and all side effects are reversible after treatment discontinuation.

2. Flares during peginterferon alfa-2b therapy could herald HBeAg response and should not be treated with additional nucleoside analogue therapy. Host-induced flares strongly predict response to therapy.

3. Peginterferon therapy is capable of enhancing HBsAg seroclearance or HBeAg seroconversion. Especially patients with genotype A benefit from peginterferon alfa-2b treatment.

4. Treatment of peginterferon alfa-2b alone or in combination with lamivudine is effective in one-third of previous non-responders to conventional IFN or lamivudine. High baseline ALT levels are the best predictors for response to therapy. Peginterferon alfa-2b therapy in patients harbouring a YMDD mutation in the polymerase gene of HBV seems less effective.

5. HBeAg relapse occurs more often after treatment with peginterferon alfa-2b and lamivudine than after peginterferon alfa-2b monotherapy. Absence of anti-HBe at the end of treatment was found to be the strongest predictor of relapse, particularly in patients treated with combination therapy.

SAMENVATTING

Infectie met het hepatitis B virus (HBV) is wereldwijd een groot gezondheidsprobleem. Meer dan 2 miljard mensen zijn in aanraking geweest met HBV en ongeveer 400 miljoen zijn chronisch drager van het virus. Chronische hepatitis B (CHB) infectie kan leiden tot levercirrose, hepatische decompensatie en hepatocellulair carcinoom (HCC). Tevens is CHB verantwoordelijk voor een geschat aantal van 1 miljoen doden per jaar.

De effectiviteit van de gevestigde behandelingen van CHB is beperkt. Behandeling met standaard interferon (IFN) is slechts effectief in 20-35%. Lamivudine moet lang gegeven worden om de respons te handhaven en leidt tot het ontstaan van therapieresistentie stammen. Verscheidene studies hebben verschillende behandelingsmogelijkheden onderzocht zoals peginterferon, adefovir of entecavir. De eerste gerandomiseerde trial in HBeAg positieve CHB patiënten met peginterferon, dat bewezen effectiever is dan standaard IFN in chronische hepatitis C, demonstreerde dat een 52 weken durende behandeling met peginterferon alfa-2b leidde tot HBeAg seroconversie in 36%, normalisatie van ALT waarden in 35% en HBV DNA waarden < 2.0 x 10^5 kopieen/mL in 32% van de patiënten aan het einde van de follow-up. De toevoeging van lamivudine verbeterde de respons percentages niet in vergelijking met alleen peginterferon alfa-2b. Standaard IFN behandeling is niet geschikt voor alle patiënten aangezien de behandeling gepaard gaat met medisch relevantie bijwerkingen, waarbij vervroegd stoppen met de behandeling genoodzaakt kan zijn. Alhoewel het bijwerkingenprofiel van peginterferon in chronische hepatitis C bestudeerd is, zijn de bijwerkingen van peginterferon in CHB nog niet onderzocht. Wij stelden de veiligheid vast van peginterferon alfa-2b alleen of in combinatie met lamivudine in 300 HBeAg positieve patiënten (hoofdstuk 2). De meest voorkomende bijwerkingen waren vergelijkbaar met die van standaard IFN. Griepverschijnselen, hoofdpijn, vermoeidheid, spierpijn, lokale reactie op de injectieplaats, buikklachten en psychiatrische symptomen waren meest frequent, maar verdwenen in het algemeen in de loop van de behandeling. Hematologische veranderingen (leukopenie, neutropenie en thrombocytopenie) werden vaak gezien, maar leidden niet tot ernstige infecties of bloedingcomplicaties. Neutropenie was de meest voorkomende oorzaak voor dosisreducties, psychiatrische bijwerkingen (depressie, psychose) waren de belangrijkste oorzaken van vervroeg stoppen met de behandeling. Reeds bestaande cirrose aan het begin van de therapie bleek de belangrijkste factor voor thrombocytopenie en (kleine) bloedingcomplicaties.

Tijdens standaard IFN behandeling en in het natuurlijk beloop van de ziekte, zijn acute opvlammingen van CHB een bekend verschijnsel. Deze 'flares' worden toegeschreven aan verhoogde T cel activiteit en de 'natural killer' cel functie en kunnen een voorbode zijn van HBeAg seroconversie. Flares tijdens therapie en follow-up van peginterferon alleen of gecombineerd met lamivudine gebeurde in 25% van de patiënten (hoofdstuk 3). De frequentie

van alle flares was vergelijkbaar tussen de verschillende HBV genotypen, maar flares tijdens therapie werden vaker gezien in genotype A dan in genotype D patiënten. Flares tijdens therapie waren geassocieerd met respons op behandeling, dit in tegenstelling tot flares optredend na behandeling. Nauwkeurige registratie onthulde 2 verschillende patronen flares; 'host-geïnduceerde' flares werden gevolgd door een sterke daling van HBV DNA waarden. 'Virus-geïnduceerde' flares werden voorafgegaan door een sterke stijging van de HBV DNA waarden. Virus-geïnduceerde waren nadelig voor de behandelingsuitkomst en traden met name op na therapie. Deze flares reflecteren waarschijnlijk de exacerbaties van CHB zoals beschreven na het stoppen van lamivudine en kunnen ernstig en levensbedreigend zijn. In tegenstelling tot virus-geïinduceerde waren host-geïnduceerde flares sterk predictief voor HBeAg verlies, 58% van de patiënten met een host-geïnduceerde flare was HBeAg negatief aan het einde van de follow-up. Dit onderstreept te meer dat volledige controle en eliminatie van het virus wordt bereikt door een sterke immuunrespons in plaats van enkel onderdrukkin van het virus.

In hoofdstuk 4 onderzochten we de frequentie van HBsAg verlies en de rol van het HBV genotype op de behandeling. HBsAg klaring of HBsAg seroconversie wordt beschouwd als het ideale doel van de behandeling, aangezien dit sterk geassocieerd is met verbeterde overleving en reductie van HCC. Spontane klaring van HBsAg is zeldzaam en behandeling met nucleoside analogen leidt eveneens zelden tot HBsAg seroconversie. In totaal waren 18 patiënten (7%) HBsAg negatief na behandeling met peginterferon aan het einde van de follow-up met geen verschil tussen de beide behandelingsgroepen. Onder HBsAg negatieve patiënten aan het einde van de follow-up had iedereen genormaliseerde ALT waarden en HBV DNA waarden < 2.0 x 105 kopieën/mL. Eenenzestig procent was HBV DNA negatief gemeten met de 'in-house Taqman PCR' (detectie limiet 400 kopieën/mL). Evenals bij HBeAg verlies was HBV genotype sterk geassocieerd met HBsAg verlies. Wij vonden HBsAg klaring in 14% van de genotype A, 9% van de genotype B, 3% van de genotype C en in 2% van de genotype D patiënten. Onder HBeAg negatieve patiënten werden respectievelijk 31%, 20%, 9% en 8% HBsAg negatief aan het einde van de follow-up.

Hoewe recente studies met nieuwe therapieopties veelbelovende resultaten laten zien, groeit de populatie van patiënten die eerder niet reageerden op standard IFN of lamivudine therapie. Tot op heden is de effectiviteit van peginterferon in deze group moeilijk te behandelen patiënten onbekend. Wij onderzochten 76 patiënten die eerder non-responder waren op standaard IFN en-/of lamivudine behandeling (hoofdstuk 5). Behandeling met peginterferon al dan niet gecombineerd met lamivudine, leidde in 35% van de eerdere non-responders op standaard IFN, 29% van de non-responders op lamivudine en 18% van de non-responders op zowel IFN als lamivudine tot HBeAg respons. Respectivelijk werd normalisatie van serum ALT waarden gezien in 24%, 41% en 14% van de non-responders. Aan het einde van de therapie had 5% van de non-responderes op IFN, 6% van de non-

responders op lamivudine en 0% van de non-responders op beide therapieën HBV DNA waarden < 400 kopieën/mL. Met name patiënten die aan het begin van de behandeling ALT waarden > 4 x de normaal waarde hadden reageerden goed op de peginterferon behandeling. Duur en dosis van de eerdere behandeling beinvloedde de uitkomst van therapie niet.

Een uitgebreidere analyse van patiënten met een YMDD mutatie wordt in hoofdstuk 6 beschreven. Deze studie liet een lage effectiviteit van peginterferon zien in patiënten die drager zijn van een YMDD mutatie van het virus. Slechts 2 patiënten (13%) converteerde naar HBeAg negativiteit gecombineerd met ALT normalisatie en HBV DNA < 2.0 x 105 kopieen/mL aan het einde van de follow-up. Deze resultaten suggereren dat de effectiviteit van peginterferon beperkt is in deze populatie. Echter, grotere studies zijn nodig om defintieve conclusies te kunnen trekken. Peginterferon in eerdere non-responders zonder YMDD mutatie lijkt gunstig effectief.

In het algemeen is de respons op standaard IFN of peginterferon behandeling in CHB patiënten duurzaam, maar terugkeer van de ziekte (relaps) na het stoppen van lamivudine wordt frequent gezien. Het effect van peginterferon al dan niet gecombineerd met lamivudine op relaps is nog steeds onbekend (hoofdstuk 7). Relaps gebeurde vaker na het stoppen van de combinatie therapie van peginterferon met lamivudine (39%) dan na het stoppen van peginterferon monotherapie. Multivariate analyse bevestigde combinatietherapie als onafhankelijk predictieve factor. Afwezigheid van anti-HBe op week 52 was eveneens een onafhankelijk factor. HBeAg negativiteit alleen lijkt derhalve minder geschikt als eindpunt van behandeling, vooral voor patiënten behandeld met combinatietherapie. De aanwezigheid van anti-HBe bij peginterferon monotherapie lijkt minder belangrijk aangezien de meerderheid van de patiënten (7 van de 10) geen relaps kreeg ondanks de afwezigheid van anti-HBe. Verder suppressie van HBV DNA waarden zou de effectiviteit kunnen vergroten, vanwege de lagere gemiddelde HBV DNA waarden in de blijvende responders vergeleken met diegene met een relapse.

Hoger lichaamsgewicht is een risicofactor voor het niet reageren op peginterferon in chronische hepatitis C patiënten. Studies met peginterferon alfa-2b, dat gedoseerd wordt op basis van lichaamsgewicht, liet geen relatie zien tussen hoger lichaamgewicht en respons, terwijl studie met peginterferon alfa-2a, dat een vaste dosis heeft, vermindere effectiviteit liet zien in zwaardere patiënten. Wij onderzochten het effect van een vaste dosis peginterferon alfa-2b van 100 µg in CHB (hoodstuk 8). Voor deze studie werden 264 patiënten in 5 gewichtscategorieën ingedeeld, ≤ 55 kg (n = 26), 55-65 kg (n = 59), 65-75 kg (n = 77), 75-85 kg (n = 58) or > 85 kg (n = 44). HBeAg response voor de corresponderende groepen was 46%, 42%, 31%, 24%, and 45%. De grote meerderheid van patiënten > 85 kg bestond uit genotype A of D. Onder de genotype A groep, reageerden patiënten > 85 kg beter dan die ≤ 85 kg (83% vs. 39%), terwijl het tegenovergestelde voor genotype D patiënten werd gevonden, 8% vs. 29% voor patiënten > 85 kg en ≤ 85 kg. In tegenstelling tot genotype D

patiënten is dosering van peginterferon alfa-2b wellicht niet nodig voor genotype A, maar toekomstige studies moeten uitsluitsel bieden.

CONCLUSIES

1. Peginterferon alfa-2b is veilig in HBeAg-positieve chronische hepatitis B. De meeste bijwerkingen worden gezien gedurende de eerste maanden van behandeling en alle bijwerkingen zijn reversibel na stoppen van therapie.

2. Flares tijdens peginterferon alfa-2b therapie kunnen een voorbode zijn van HBeAg respons en dienen niet met additionele nucleoside analogen behandelt te worden. Host-geïnduceerde flares hebben eens sterk voorspellende waarde op respons.

3. Peginterferon is in staat tot HBsAg klaring en HBsAg seroconversie te vergroten. Met name patiënten drager van het genotype A profiteren het meest van peginterferon alfa-2b therapie.

4. Behandeling van peginterferon alfa-2b al dan niet in combinatie met lamivudine is effectief in eenderde van eerdere non-responders op standard IFN of lamivudine. Hoge aanvangswaarden van ALT zijn de beste voorspellers voor response op therapie. De effectiviteit van peginterferon alfa-2b in patiënten die een YMDD mutatie dragen lijkt afgenomen.

5. HBeAg relaps gebeurt vaker na het stoppen van de combinatie van peginterferon alfa-2b met lamivudine dan na peginterferon alfa-2b alleen. Afwezigheid van anti-HBe aan het einde van de therapie was de sterkste voorspeller op relaps, vooral in patiënten behandelt in de combinatietherapie.

APPENDIX

The HBV 99-01 Study Group includes the following investigators:
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CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 24 december 1978 te Veendam. Na het behalen van zijn V.W.O. diploma aan het "O.S.G. Winkler Prins" in 1997, studeerde hij Technische Bedrijskunde aan de Rijksuniversiteit Groningen. In 1998 werd hij ingeloot voor de studie Geneeskunde aan de Erasmus Universiteit Rotterdam. Al vroeg in deze periode ontstond de interesse in maag-, darm- en leverziekten, wat resulteerde in een afstudeeronderzoek naar de bijwerkingen van peginterferon bij chronische hepatitis B. Aansluitend werkte hij tot december 2005 als wetenschappelijk onderzoeker op de afdeling Maag-, Darm- en Leverziekten van het Erasmus MC. Onder leiding van zijn co-promotor en tevens promotor prof. dr. H.L.A Janssen vervolgde hij zijn onderzoek naar de effecten van peginterferon behandeling bij chronische hepatitis B patiënten. In februari 2006 startte hij met zijn co-schappen om de opleiding tot basisarts te voltooien. Tijdens zijn promotieonderzoek volleybalde hij onder andere bij eredivisionist "Rijnmond Volleybal". Hij woont samen met Sanne Ros.

BIBLIOGRAPHY

1. Flink HJ, Sprengers D, Hansen BE, et al. Flares in chronic hepatitis B patients induced by the host or the virus? Relation to treatment response during Peg-interferon {alpha}-2b therapy. Gut. 2005 Nov;54(11):1604-9.

2. Flink HJ, van Zonneveld M, Hansen BE, et al. Treatment with Peg-interferon alpha-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype. Am J Gastroenterol. 2006 Feb;101(2):297-303.

3. Leemans WF, Flink HJ, Janssen HL, et al. The effect of pegylated interferon-alpha on the treatment of lamivudine resistant chronic HBeAg positive hepatitis B virus infection. J Hepatol. 2006 Mar;44(3):507-11.

4. van Zonneveld M, Flink HJ, Verhey E, et al. The safety of pegylated interferon alpha-2b in the treatment of chronic hepatitis B: predictive factors for dose reduction and treatment discontinuation. Aliment Pharmacol Therap. 2005 May1;21(9): 1163-71.

5. van Zonneveld M, Zondervan PE, Cakaloglu Y, et al. Peg-interferon improves liver histology in patients with HBeAg-positive chronic hepatitis B: no additional benefit of combination with lamivudine. Liver Int. 2006 May;26(4):399-405.

6 Hajo J. Flink, Bettina E. Hansen, Monika van Zonneveld, et al. Is weight-based dosing treatment necessary for Peg-interferon alpha-2b therapy in HBeAg-positive chronic hepatitis B? (submitted)

7, Hajo J. Flink, Bettina E. Hansen, E. Jenny Heathcote, et al. Successful treatment with Peginterferon alfa-2b of HBeAg-positive HBV non-responders to standard Interferon or Lamivudine (in press Am J Gastro)

8. Hajo J Flink, Erik HCJ Buster, Bettina E Hansen, et al. Relapse after treatment with peginterferon alfa-2b alone or in combination with lamivudine in HBeAg positive chronic hepatitis B (submitted)

LIST OF ABBREVIATIONS

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMI	body mass index
cccDNA	covalently closed circular DNA
CHB	chronic hepatitis B
CI	confidence interval
DNA	Deoxyribonucleic Acid
HCC	hepatocellular carcinoma
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HDV	hepatitis D virus
HIV	human immunodeficiency virus
IFN	interferon
mL	mililiter
NA	nucleoside analogue
NR	non-responder
ORF	open reading frame
PCR	polymerase chain reaction
PEG	polyethylene glycol
Peg-IFN	pegylated interferon
RR	relative risk
RNA	ribonucleic acid
SAE	serious adverse event
SD	standard deviation
SSRi	specific serotonin reuptake inhibitor
U	units
ULN	upper limit of normal
WBC	white blood cell count
WHO	World Health Organisation
YMDD	thyrosine methionine aspartate aspartate